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OCTOBER, 1929

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PLANT PHYSIOLOGY

OCTOBER, 1929

COMPOSITION AND GROWTH INITIATION OF DORMANT BARTLETT PEAR SHOOTS AS INFLUENCED BY TEMPERATURE

F. E. Gardner

(WITH NINE FIGURES)

Introduction

Considerable attention has been given in the past to methods of shortening the normal rest period of plants. The agents used to break the rest have included warm baths, nutrient salts, narcotics, anaesthetics, freezing and thawing, wounding, exposure to low temperatures, and other means of stimulation. However, in spite of the variety of means available for awakening the plant, we apparently have no convincing evidence as to how any of these agents bring about this renewal of growth. While it is not certain that all treatments which break the rest period act in a similar manner, it does not appear altogether unlikely that there may be a general principle involved: that a particular internal condition, within limits, must be attained before growth can take place.

Exposure to low temperature has long been recognized and employed as a successful means of terminating the rest period. The full significance of this natural means, however, is usually not appreciated, since in most temperate climates the season of dormancy is always accompanied by relatively low temperature, and the cold weather is commonly regarded as the cause for the lack of growth, whereas, it is probably the necessary antecedent to the growth of many plants.

In some regions, as in southern California, the winter months are not always accompanied by weather cold enough to bring about a normal spring awakening of all trees. This condition in the springs of 1904, 1924, and 1926, following unusually mild winters, prevailed to the extent that there were many acres of fruit trees completely dormant or growing indifferently

from only a few buds, when they should normally have been in full leaf. Crop failures resulted in the varieties which exhibited this trouble most markedly. HORNE, WELDON and BABCOCK (18), who have described this condition, are of the opinion that the trouble occurs every year in southern California to a limited extent and that it is not peculiar to California but is of general occurrence in very mild climates.

Various theories to account for the rest period and its termination have invoked changes in permeability, enzyme activity, food reserves, hydrogen-ion concentration, and other possible factors. One of the most prominent has been the question of organic food supply, with its direct relation to enzyme activity. Since but little is known concerning the nature of the chemical changes accompanying the breaking of the rest, the present work was undertaken to ascertain the effect of temperature on some of the constituents which are ordinarily considered important in the metabolism of the plant and which may bear some relation to the termination of the rest period.

Review of literature

The literature pertaining to the rest period abounds in theories concerning the nature of the rest and in methods for its termination. These have been amply reviewed by HOWARD (19), WEBER (38), and APPLEMAN (2). The literature here referred to is concerned chiefly with the chemical changes accompanying the entrance into or the exit from the rest, and includes investigations on seeds, tubers, and woody tissues.

"After-ripening," which is characteristic of most seeds, and which is necessary in a greater or less degree for germination, is analogous to the rest period of woody plants. In some cases, delay in seed germination is due to the inhibiting nature of the seed coat, as CROCKER (7) has shown; but SHULL (36) has demonstrated that the delay is not necessarily entirely a seed coat effect, but may be due to a lack of stimulation of the embryo, as in the case of *Xanthium* seeds. DAVIS and ROSE (8), working with seeds of *Crataegus mollis,* have pointed out that an exposure to low temperature is an effective means of stimulating the embryo of this seed. Using the same temperature, 5–6° C., found by DAVIS and ROSE to be most suitable for the after-ripening of *Crataegus* seeds, ECKERSON (12) followed some of the metabolic changes occurring in the embryo of this seed during the after-ripening process. The food, she states, is stored in the form of fatty oils. There is also present considerable lecithin but no starch nor sugar. The initial change appears to be an increase in acidity, correlated with an increased water holding power and a heightened activity of catalase and peroxidase. She found that the after-ripening process could be greatly shortened by treating the embryos with dilute hydrochloric, butyric, or acetic acids.

PACK (**29**), in studying the after-ripening and germination of *Juniperus* seeds found that this seed, too, has a dormant embryo which must go through a series of changes before germination. For after-ripening, 5° C. seemed to be most favorable. The changes taking place at this temperature include an increase in hydrogen-ion concentration of the embryo, a dispersion of stored fats, a decrease in fats and proteins, an increase in sugar and amino acids, and a doubling of catalase activity. A later paper (**30**) appears to summarize PACK's rather inclusive view of the after-ripening process: "Accumulation of cell building materials: acids, phosphatides, active reducing substances, soluble sugars, pentoses, amino acids, soluble proteins, and other nitrogenous compounds; the accumulation of enzymes; the dispersion of materials; and the transformation of storage materials. This rapid accumulation of simple plastic cell materials, coupled with minimum respiration and combustion of materials, probably forces the dormant organs to activity. One thus sees the awakened active organ as a very unstable structure made up of many unstable compounds. If these changes are not the basis of the after-ripening process, they are found to accompany the after-ripening process."

A number of workers have demonstrated the importance of sugars for seed germination. IVES (**20**) tried various means of forcing seeds of *Ilex opaca*—a seed very difficult of germination; but no success was encountered with acids, bases, salts, electrical stimulation, etherization, light, oxygen, or enzymes. However, low temperature and treatment with a 5 per cent. dextrose solution were successful. Dextrose was reported to increase the germination from one in a million seeds up to sixty per cent. germination. KNUDSON (**22**) also has found sugars to be of assistance in the germination of orchid seeds. In this case fructose appeared to be more favorable than glucose.

The chemical changes found by various workers to occur in potato tubers are more consistent than those reported for seeds. MÜLLER-THURGAU (**25**) found an increase in the sugar content of tubers when they were subjected to low temperature. The tubers never formed sugar when frozen rapidly, but if their temperature was lowered slowly to −5° C. during fourteen days, the sugar content on the average increased from 0.3 per cent. to 0.92 per cent. At the same time more starch had disappeared than was necessary to account for the increase in sugar. He found no appreciable difference in the nitrogen determinations of chilled and unchilled potatoes. MÜLLER-THURGAU and SCHNEIDER-ORELLI (**26**) employed the warm bath method to hasten the germination of potato tubers and lily-of-the-valley bulbs. The warm bath, like low temperature, heightened the sugar content; but these workers do not attribute the hastened growth in this case to the

increased sugar, but rather to some effect of high temperature on the protoplasm.

APPLEMAN (**2**) reports an increase in the reducing sugar content of potato tubers under the influence of low temperature. He compared two sets of tubers, one held constantly at a growing temperature, and the other at a variable low temperature. Analyses of these two sets were made from time to time throughout the storage period. No change was found in the amounts of diastase or invertase to explain the carbohydrate changes. He states that shortening of the rest in this case did not involve protein hydrolysis; for he found no appreciable differences between the two sets in the following nitrogen fractions—proteose nitrogen, peptones, amino acids, and amides. The various forms of phosphorus, too, remained constant until after germination had begun. APPLEMAN concludes that the rest period of potato tubers is not a firmly fixed and hereditary process, as many others (**21**) believe to be the case regarding this period in general.

BUTLER (**4**), HOPKINS (**17**), and DENNY (**9**) have also noted the increase in sugars in potato tubers following treatments which terminate the rest period. DENNY (**9**), in agreement with the findings of MÜLLER-THURGAU (**25**) and APPLEMAN (**2**), reports no consistent differences in various nitrogen constituents between awakened and dormant tubers.

Although low temperature, as an agent which breaks the rest period, has often been reported to increase the sugar content, ROSA (**34**) failed to obtain any shortening of the dormant period by low temperature. A high temperature (28–30° C.) did, however, have a marked accelerating effect on growth. LOOMIS and EVANS (**24**) suggest that in vegetative organs such as corms, tubers and woody twigs, which contain stored starch, the breaking of the rest period by ethylene chlorhydrin, ether and similar compounds is connected with the hydrolysis of the starch. Bulbs, on the other hand, which contain little or no starch, are not affected by treatment with these compounds.

Concerning the effect of temperature on the chemical changes taking place in woody tissues, less definite information is available than in the case of seeds and tubers. Perhaps the explanation is to be found in the relative ease of experimentation with these smaller organs. Although there are considerable chemical data indicating the seasonal changes in parts of trees throughout the year, it is not known what course these changes would follow under different temperature conditions.

PFEFFER, NEGER, DRUDE, JOST, JOHANNSEN, SPÄTH, MAGNESS, and others (**21**) do not associate the rest period with chemical changes brought about by temperature effects, but believe that the yearly periodicity is the result of hereditary rhythm. KLEBS (**21**), on the other hand, considers it to be

the result of the relation of the plant to the outer world. The rest period begins, he believes, if, through a decrease in one or more factors—temperature, moisture, nutrient salts, etc.—the growth activity is lessened while assimilation continues until the accumulation of organic materials brings about an inactivation of the enzymes. HOWARD (**19**) who also favors this enzyme concept believes that all the phenomena connected with the entire resting phase are closely associated with the work of enzymes. He has demonstrated an increase in enzyme activity following various treatments which break the rest period. COVILLE (**6**), who also associates the breaking of the rest with the work of enzymes, has advanced a more specific view. He proposes that the starch stored in the tissues is separated from the enzymes by cell membranes; but, as the result of chilling, the vital activity of the membranes is weakened, and the enzyme "leaks through" to the starch and converts it into sugar. The high osmotic pressure resulting from the increased sugar initiates the growth of the buds.

While FISCHER (**13**) concludes that periodicity of growth is conditioned by a periodicity of the processes of food changes, he believes that these changes in turn rest upon an hereditary periodicity of certain properties of protoplasm. He followed the starch changes in the tree throughout the dormant season and found a starch maximum from leaf fall until November, then a decrease during the winter months, followed by a secondary maximum in April. PERETOLCIN (**31**) found that in the oak the period of greatest starch content corresponded to the period of deepest rest. Working with *Quercus, Fraximus, Ulmus,* and *Tilia,* he reports a gradual conversion of starch into fats as the rest period draws to a close. WEBER (**38**) also reports an inverse relationship between starch and fat content in a number of species. TUTTLE (**37**), working chiefly with *Linnaea,* an evergreen species, claims that exposure to high temperature (20° C.) caused a conversion of oil into starch in the leaves, while a reconversion into oil was brought about by an exposure for eight days to 0° C. She states that in northwestern Canada, where chilling weather sets in early in the fall, the leaves of most evergreen trees are destarched by October and that they then contain large amounts of oils.

NIKLEWSKI (**27**), using excised branches of *Tilia, Prunus,* and *Betula,* quantitatively determined the amount of fats present in the branches before and after exposure to various temperatures from 1° to 22° C., and concluded that the fat content bears no relation to temperature. He does, however, report an increased sugar content in both bark and wood under the influence of low temperatures. POJARKOVA (**32**) found a relationship between the inversion of starch into sugar and the breaking of the rest, but reports complete absence of fats in the species investigated—*Berberis, Lonicera,*

Amelanchier, Acer, and *Corylus.* Evidently the conversion of starch to fats, sometimes reported to take place, is not of universal occurrence in all trees, nor does it appear to be indispensable to the breaking of the rest period.

KLEBS (**21**) believes that the rest period may begin because of lack of mineral nutrients to the buds. RIPPEL (**33**), however, calls attention to the fact that nitrogen and other elements are moved back from the leaves to the buds just before leaf fall, and that this increased mineral supply to the buds comes at a time when the rest period is beginning. While it is his opinion that the rest is something more than a matter of mineral supply, yet he shows the importance of mineral nutrients for continued development of the buds once their growth has been initiated.

ABBOTT (**1**) determined some of the chemical changes taking place at the beginning of the rest period in the tips of one-year-old peach seedlings and in the tips and bark of Grimes apple trees. The analyses show an increase during August, September, and October in sugars, starch, and total carbohydrates. The hydrogen-ion concentration of the expressed sap decreased in the fall and remained relatively low throughout the dormant season until growth began in the spring, at which time it again increased.

BORESCH (**3**) has shown that a high temperature coupled with insufficient oxygen leads to a considerable increase in free organic acids in plant tissue. This, he believes, is the effective action of the warm bath as a method of breaking the rest. The increased production of acids and the resultant change in pH of the protoplasm, may hasten bud development.

In regard to changes in sap concentrations, LEWIS and TUTTLE (**23**), working with sap expressed from evergreen leaves, found an increasing osmotic pressure during the winter and a subsequent falling off in the spring. The electrical conductivity during the same period remained fairly constant, indicating that the change in osmotic pressure was due to non-electrolytes. Sugar estimations on the sap followed in general the osmotic changes. GAIL (**14**) also reports that the density in the sap of leaves from pine and broad leaved evergreen shrubs increases during the winter months and reaches a maximum in late January and February.

This brief review of investigations concerning the changes associated with the breaking of the rest period serves to indicate the fragmentary and inconsistent nature of the evidence relative to this very important phase in the life of the plant.

Experimental material and methods

It is not meant in this section to include unnecessary details of procedures with which investigators are perfectly familiar. However, sufficient

details should be recorded to enable the reader to evaluate for himself the results obtained. This is particularly true of the analytical methods where a number of deviations from the usual procedures occur and where some justification of the methods used is necessary.

Material

Five-year-old Bartlett pear trees, grown in Berkeley, were used for this problem. These trees were heavily pruned in the early spring so that by fall they had produced an abundance of straight upright shoots, two and a half to three feet in length. These shoots constituted an unusually uniform group of material for analytical purposes.

On October 26, twenty-four trees were defoliated and dug from the orchard, as much of the root system being preserved intact as it was practicable to accommodate in the boxes of sand in which the trees were then planted. Eight of the trees, in boxes of moist sand, were placed in cold storage at a temperature of 2° C. A similar set of eight was maintained in the greenhouse, the temperature of which was controlled so that it never dropped below 16° C. The third set of eight was placed outside the greenhouse, thereby exposing the trees to the fluctuations of temperature characteristic of the winter season in Berkeley.

Sampling

Each sample collected for analysis consisted of twenty-four shoots, selected from the eight trees of the set with reference to uniformity of length, size, and position on the tree. The upper two feet of each shoot, constituting about three-fourths of its total length, were used for analysis.

The first three collections of shoots, dated September 11, October 2, and October 24, were taken from the selected trees before the trees were dug from the orchard in order to ascertain the normal trend of changes before subjection to different temperatures. After the trees had been dug and moved to the three temperature conditions previously stated, collections of shoots from the three sets of trees were made approximately every two weeks throughout the winter period in order to follow the chemical changes taking place in each set under its particular temperature condition.

In all cases bark was separated from wood for individual determinations. The samples were weighed and rapidly dried in a fan-ventilated oven at 78° C. and when dry, were re-weighed for moisture determinations and ground to a fine powder, most of the particles of which were able to pass a 100-mesh screen.

Alcoholic extraction

5-gm. portions of the dry, powdered samples, were extracted in the Soxhlet apparatus with 95 per cent. alcohol to which had been added a small

amount of sodium carbonate. The extract was then entirely freed from alcohol by means of a hot air blast, taken up in warm water, filtered, and made up to a volume of 200 cc. with distilled water.

Analytical methods

Biological methods for the determination of the carbohydrates have been used as far as practicable. The use of enzymes instead of acids in the procedures involving hydrolysis has made "clearing" of the solutions unnecessary. Because of the methods employed it is believed that the fractions reported here represent rather closely the substances named—that the fraction termed "hexose sugar" represents only hexose sugar, and similarly for the other carbohydrate contituents.

Determination of reducing value.—The Shaffer and Hartmann (35) iodometric method for the determination of reducing value was employed with minor variations. In all cases the cuprous oxide precipitate was collected and washed on an asbestos mat in a Gooch crucible before it was dissolved and titrated. This process eliminated substances which may in themselves have no power of reducing Fehling's solution, but which might form addition or substitution products with iodine and thereby vitiate the titration. This precaution seems to be necessary in the case of pear tissues which contain large amounts of arbutin.

Total free reducing substances were determined on a 50 cc. aliquot of the uncleared solution.

Hexose sugar.—To a 50 cc. aliquot of the extract, a small cube of Fleischmann baker's yeast (approximately 0.3 gm.) and 4 cc. of 95 per cent. alcohol were added. The solution was shaken to get the yeast into suspension and then incubated 18–24 hours at 37° C. An occasional agitation during that period facilitated the fermentation of the hexose. It was found that yeast, by means of its emulsin, would split arbutin, which, as has been mentioned, is present in pear tissues, and particularly in the bark, in relatively large amounts. In preliminary work, this often resulted in a higher reducing value after fermentation than before, due to the hydrolytic product, quinol, not fermented by the yeast. The addition of alcohol checked the activity of the yeast emulsin, but did not interfere with complete hexose fermentation. The difference between the total free reducing substances and the reducing value after fermentation represents hexose sugar.

Non-hexose free reducing substances.—This fraction is represented by the reducing value obtained after all hexose material had been fermented away by the yeast.

Sucrose.—The increase in reducing value after invertase inversion is recorded as sucrose. Wallerstein's invertase scales were employed in this

determination, using 5 cc. of a 1 per cent. solution of the scales to 50 cc. of the plant extract.

It was found that "Difco" invertase was unsatisfactory when added to uncleared solutions, for a precipitation invariably resulted and often a decrease in reducing value instead of the expected increase. Presumably tannins or other substances capable of reducing Fehling's solution were precipitated by "Difco" invertase. The scales appear to be free from this objection. Incubation was carried out at 37° C. over night. In this case, also, 4 cc. of 95 per cent. alcohol was added to the solutions with the enzyme, since the invertase was not found to be free from emulsin which would otherwise split some of the arbutin present. The invertase scales have a small blank for which correction was necessary.

Arbutin.—For hydrolysis of this rather large fraction, the enzyme emulsin was used. Commercial emulsin seemed open to the same objection stated for "Difco" invertase, namely, that when added to uncleared solutions, precipitation resulted. A very active emulsin was prepared from sweet almonds, using the method described by ONSLOW (**28**). This preparation was not, however, free from invertase, and for this reason it was used in conjunction with invertase scales in order to be certain that complete inversion of the sucrose was effected as well as hydrolysis of the arbutin. Pure arbutin could be completely hydrolyzed by this emulsin within thirty-six hours at 37° C. The arbutin reported represents the difference between the reducing value after invertase inversion and that obtained after the use of emulsin plus invertase.

Starch.—To the sample residue, after extraction with alcohol, boiling water was added and the mixture allowed to boil for fifteen minutes on a hot plate. After cooling, 5 cc. of saliva and NaCl sufficient to make a 1 per cent. solution were added. Incubation was carried out at 40° C. for twenty-four hours. Continual agitation, by means of a shaker, facilitated the digestion.

Saliva was used for the digestion, in preference to Taka diastase, as being more specific for starch, since Taka diastase contains such a mixture of enzymes. Moreover, saliva has a zero blank with Fehling's solution, whereas, Taka diastase has a large one which becomes still larger if followed by acid hydrolysis.

Due to the presence of water-soluble acid-hydrolyzable material, not hydrolyzed by amylase and which therefore does not properly belong to the starch fraction, a factor, instead of the usual acid hydrolysis, was used to compute starch as glucose. The experimental curve (fig. 1), was constructed from data obtained by the use of potato starch and saliva under the same conditions of incubation given the plant samples. The reducing value in terms of cc. of thiosulphate after treatment with saliva is plotted

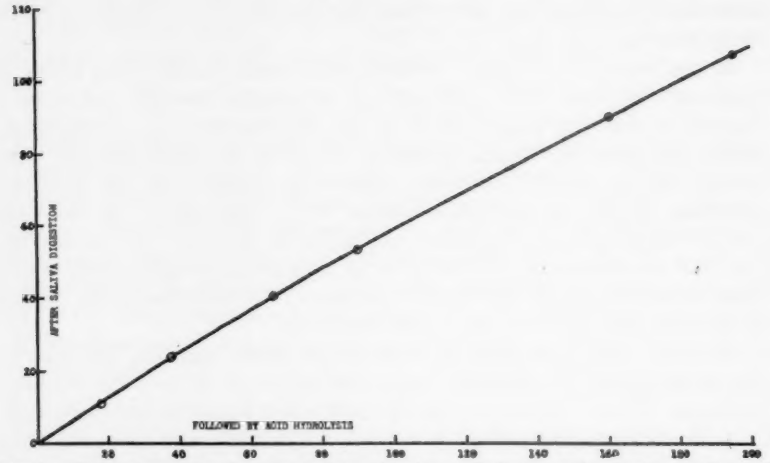

FIG. 1. Conversion curve for starch determinations. Reducing value in terms of cc. of thiosulphate after saliva digestion is plotted against value after acid hydrolysis of pure potato starch.

against the value obtained after acid hydrolysis of aliquots of the same samples. Using saliva in a constant amount, the factor, by which the reducing value after treatment with saliva must be multiplied in order to convert it into terms of glucose, is seen from the curve and from table I to increase as the amount of starch increases.

TABLE I

REDUCING VALUE OF INCREASING AMOUNTS OF POTATO STARCH AFTER SALIVA DIGESTION AND ACID HYDROLYSIS

AFTER SALIVA DIGESTION (THIOSULPHATE)	FOLLOWED BY ACID-HYDROLYSIS (THIOSULPHATE)	FACTOR
cc.	*cc.*	
11.6	17.8	1.534
23.9	37.7	1.575
40.5	66.0	1.63
53.6	89.0	1.66
90.5	160.0	1.77
107.0	195.0	1.82

Water-soluble polysaccharides other than starch.—After the determination of starch, a 50 cc. aliquot of the solution already digested by saliva was hydrolyzed with a 3 per cent. HCl for an hour in a boiling water bath

and then neutralized with NaOH. The difference between the reducing value after acid hydrolysis and the value from the starch conversion curve represents the water soluble polysaccharides.

Total nitrogen.—This determination was made by the Kjeldahl-Gunning method, using 5 gm. of dry sample.

Amide nitrogen.—This nitrogen fraction was determined by the standard method of SACHSSE, using 10 gm. of dry sample.

Freezing point depression.—These determinations were made with a Beckmann thermometer, on the sap immediately after its expression. The press used for obtaining the sap exerts a pressure on the sample of 870 kilograms per cm.^{2}, and the expression was continued until all the sap obtainable with this apparatus had been collected.

Electrical conductivity.—The conductivity of the expressed sap was determined by the standard method of Kohlrausch at 25° C.

Free organic acids and esters.—These were determined on 10 cc. portions of expressed sap which had been preserved in alcohol. An excess of 0.1 N NaOH was added and saponification carried out in a boiling water bath. The excess base was then titrated with standard acid, using phenolphthalein as an outside indicator. The natural color of the solution made detection of the end-point difficult; hence, the results are given as only approximate. Most of the acid found was combined, but it was not ascertained whether the acids of the fresh sap are largely combined, or whether esterification had taken place during the storage of the samples in alcohol.

Results

GROWTH RESPONSES

Cessation of the resting condition is not abrupt, but rather a gradual transition. The growth responses of orchard and storage trees reflected this gradual dissipation of the rest; and for this reason a sharp line demarcating the resting from the active condition can hardly be drawn through a series of frequent observations.

The experimental trees kept outdoors during the winter began growth in the spring at the normal awakening time. Digging of the trees in the fall evidently did not influence their time of exit from the rest period. Shoots were removed from these trees at frequent intervals throughout the winter and placed in jars of water in the warm greenhouse in order to follow the progress of the rest period. Shoots cut from the outdoor trees previous to December 7 exhibited practically no activity. Little chilling weather had been experienced up until this time. The collection of January 5 made a little growth after about three weeks in the greenhouse. This consisted chiefly of the unfolding of flower buds. Each successive collection

from these trees showed a quicker and more complete response than the last. By February 4 very fair response was secured within a week or ten days after transfer to the greenhouse, and it was judged that the rest period of the shoots from the orchard trees was over at this time.

Shoots from storage trees were capable of growth much earlier than those from the orchard set. By November 23, after four weeks in cold storage, a few flower buds opened within three weeks following the removal to the greenhouse. Here again each successive collection responded better and more quickly than the preceding one. By January 19, all buds showed considerable activity within four days after transfer to the greenhouse.

The absence of growth from the greenhouse trees was very striking. Although given warm temperature and abundance of moisture throughout the year these trees remained dormant, except for little tufts of growth around the stubs left after the removal of shoots. This growth, which was obviously the effect of wound stimulus, was decidedly localized. Except for a few terminal buds which now and then pushed out into a weak growth, there was no evidence of awakening on the part of the greenhouse trees after eleven months of external conditions favorable for growth. Shoots removed from these trees and placed in water also failed to awaken.

Temperature conditions in Berkeley

Figure 2 shows the temperature conditions in Berkeley to which the

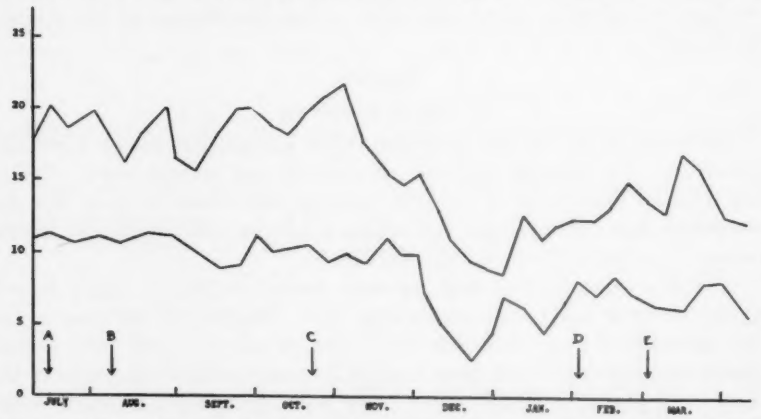

Fig. 2. Daily maximum and minimum temperatures in Berkeley, 1926–1927, averaged weekly. A, cessation of terminal growth. B, bark beginning to stick. C, trees dug. D, rest period over. E, buds swelling.

orchard trees were exposed. The state of activity of the trees is also indicated at intervals. Although the minimum temperature during the

winter months averages about 7° C., the lowest point in the curve being for the last week of December with an average minimum temperature of 2.5° C., yet this degree of chilling is apparently sufficient to bring about a normal spring awakening. Since the trees kept in the greenhouse, where the minimum temperature was 16° C., failed to awaken, it is apparent that the least chilling temperature which can break the rest period of the Bartlett pear tree lies roughly somewhere between 2.5° and 16° C. The length of exposure to a given temperature is probably important also. A longer exposure to only a moderately low temperature may be as effective in breaking the rest period as a shorter exposure to a lower temperature.

From the temperature curve it can be seen that the resting condition of the shoots was attained before the advent of chilling weather. This is in agreement with HOWARD's view (**16**), that the beginning of the rest is due wholly to inner causes.

ANALYTICAL RESULTS

Throughout the following tables and graphs the designations "orchard," "greenhouse," and "storage," respectively, refer to shoots taken from the trees maintained in the open with its uncontrolled temperature conditions, in the greenhouse where the temperature was never lower than 16° C., and in the storage at a constant temperature of 2° C. The first three collections of "orchard" material were made before the trees were dug; after which time, October 27, all trees were transplanted into boxes of sand and exposed to the temperature conditions stated for each set.

TABLE II

TOTAL MOISTURE, EXPRESSED IN PERCENTAGE OF FRESH WEIGHT

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
September 11	62.5			51.2		
October 2	59.9			45.3		
October 24	59.1			45.2		
November 4	59.0	59.1	60.3	45.8	47.8	46.5
November 23	59.4	58.6	59.8	46.0	44.9	46.8
December 7	58.0	58.0	58.3	45.6	44.8	46.9
December 23	58.3	63.0	58.5	47.0	46.2	47.4
January 5	58.6	59.4	58.2	46.8	46.9	47.1
January 19	58.6	58.7	58.4	47.8	47.0	47.6
February 4	57.6	58.7	58.2	47.8	47.5	46.3
February 17	58.3	59.2	58.1	48.6	47.3	45.6
March 7	57.5	60.0	60.0	48.0	47.7	47.2

The moisture content throughout the period of experimentation is seen from table II to remain fairly uniform in all three sets of trees. Apparently a marked change in water content is not necessarily associated with the cessation of the rest period, nor with chemical transformations which take place under the influence of temperature. Because of the uniformity in moisture, expression of the following chemical data on a dry weight basis seems reliable for comparative results.

TABLE III

HEXOSE SUGAR, EXPRESSED AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
September 11	40.5			17.2		
October 2	41.4			24.5		
October 24	60.3			24.5		
November 4	59.0	78.0	95.0	24.5	23.0	21.0
November 23	53.0	72.0	108.0	24.5	19.0	39.0
December 7	70.0	60.0	99.0	27.0	21.0	36.0
December 23	76.0	38.0	100.0	24.5	16.0	43.0
January 5	87.0	50.0	118.0	26.0	21.0	50.0
January 19	103.0	53.0	112.0	27.0	19.0	37.0
February 4	123.0	55.0	123.0	23.0	16.0	35.0
February 17	99.0	70.0	103.0	21.0	15.0	25.0
March 7	72.0	58.0	93.0	23.0	14.0	23.0

As regards the effect of temperature on the hexose content (table III and fig. 3) it is to be noted that the largest accumulation occurs under the condition of lowest temperature, while in the greenhouse trees hexose fails to increase.

The non-hexose free reducing substances indicate no constant differences between the three sets of trees. While it is not definitely known what constitutes this fraction, it may include among other substances, tannins, pentoses, and quinol. The variations in amount appear to be unaffected by temperature and to bear no relation to the changes accompanying the breaking of the rest period.

The effect of low temperature on the sucrose content is very similar to its effect on hexose sugar, although even more marked (table V and figure 4). Figure 5 expresses total sugars in each of the three sets of trees.

Arbutin, which assumes relatively large proportions in pear bark, appears to play no part, at least by variations in its amount, in the mechanism of growth-releasal. Under the influence of temperature it remains practi-

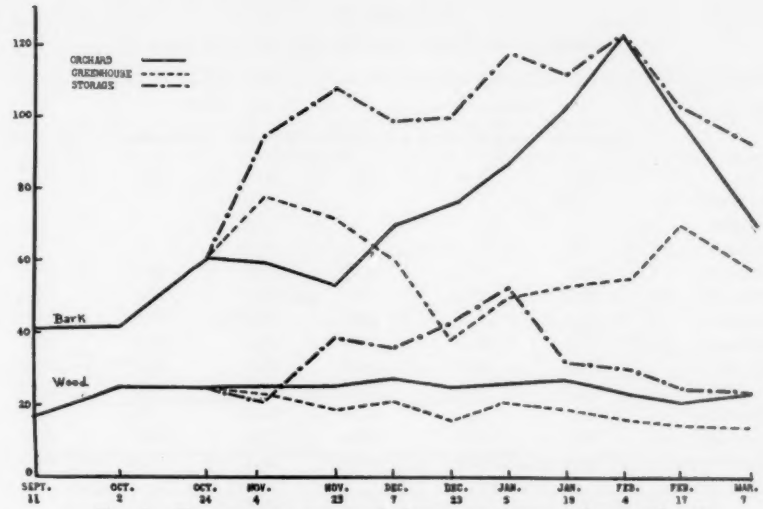

FIG. 3. Hexose sugar, as mg. of glucose per 5 gm. of dry sample.

cally unaffected (table VI). Some writers have postulated that glucosides are closely involved in the respiratory mechanism. If this is true it is interesting that arbutin remained so uniform under these widely different temperature conditions, which presumably resulted in marked differences in respiration.

TABLE IV

NON-HEXOSE FREE REDUCING SUBSTANCES, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
September 11	120			13		
October 2	120			11		
October 24	116			7		
November 4	126	110	127	19	8	11
November 23	126	95	121	12	8	15
December 7	117	106	114	13	11	12
December 23	148	110	130	9	13	11
January 5	144	112	123	16	9	11
January 19	133	118	127	21	9	11
February 4	128	136	127	23	11	12
February 17	124	123	145	15	11	11
March 7	142	134	145	19	11	15

TABLE V

SUCROSE, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
September 11	5.0			6.0		
October 2	7.5			5.0		
October 24	9.0			7.0		
November 4	28.0	13.0	24.0	11.0	13.0	25.0
November 23	29.5	10.0	54.0	13.0	16.0	29.0
December 7	43.0	17.0	90.0	14.0	10.0	41.0
December 23	43.0	10.0	118.0	15.0	15.0	42.0
January 5	34.0	19.0	114.0	20.0	10.0	33.0
January 19	52.0	19.0	120.0	27.0	10.0	33.0
February 4	41.0	24.0	106.0	25.0	6.0	33.0
February 17	56.0	19.0	100.0	26.0	12.0	37.0
March 7	43.0	17.0	120.0	18.0	11.0	36.0

The changes in starch content at different temperatures are shown in table VII and figure 6. The starch increase in the fall, while the leaves were still on the trees, and the decrease from then on, characterize the curves. The greatest drop is seen in the storage trees—the same trees which showed the largest sugar increase. It is apparent, however, that the

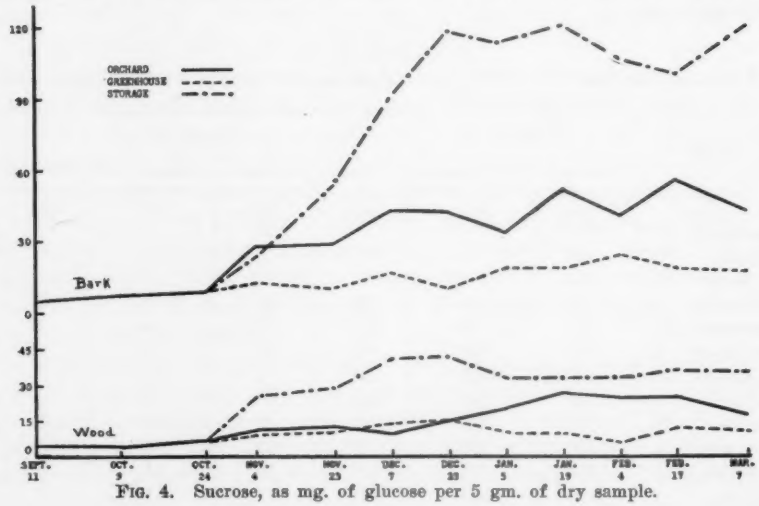

FIG. 4. Sucrose, as mg. of glucose per 5 gm. of dry sample.

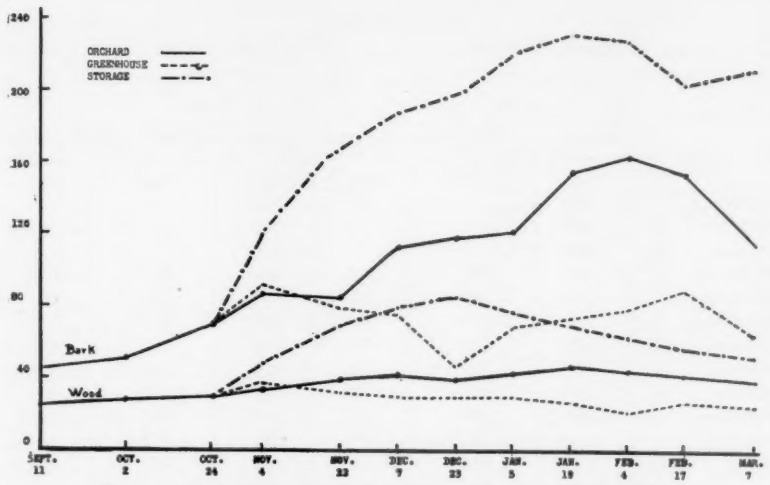

FIG. 5. Total sugars, as mg. of glucose per 5 gm. of dry sample.

decrease in starch is greater than is necessary to account for the increase in sugars. While this discrepancy might be accounted for entirely by respiration, there is still the possibility of starch conversion into some constituent not included in the analyses.

The fraction designated "water-soluble polysaccharides other than starch" is reported for pear bark only (table VIII). If present at all in

TABLE VI

ARBUTIN, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
September 11	227			39		
October 2	241			44		
October 24	245			35		
November 4	237	242	245	39	40	36
November 23	240	245	245	31	36	41
December 7	245	217	246	29	31	26
December 23	226	242	245	39	37	42
January 5	226	237	222	40	42	38
January 19	217	235	226	35	48	36
February 4	214	192	222	41	42	40
February 17	226	203	230	45	37	40
March 7	237	202	222	39	36	37

TABLE VII

STARCH, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
September 11	136			317		
October 2	185			505		
October 24	268			620		
November 4	224	210	163	684	705	615
November 23	185	200	108	625	676	560
December 7	166	200	90	625	615	520
December 23	163	200	80	596	573	495
January 5	154	186	80	570	548	476
January 19	145	198	83	517	515	455
February 4	133	175	80	470	476	437
February 17	111	175	71	455	480	446
March 7	111	169	71	461	476	420

the wood its amount is no greater than the experimental error of the starch determination. It is seen to be an erratic quantity, showing no particular trend and disclosing no relationship to the problem of the rest period. It

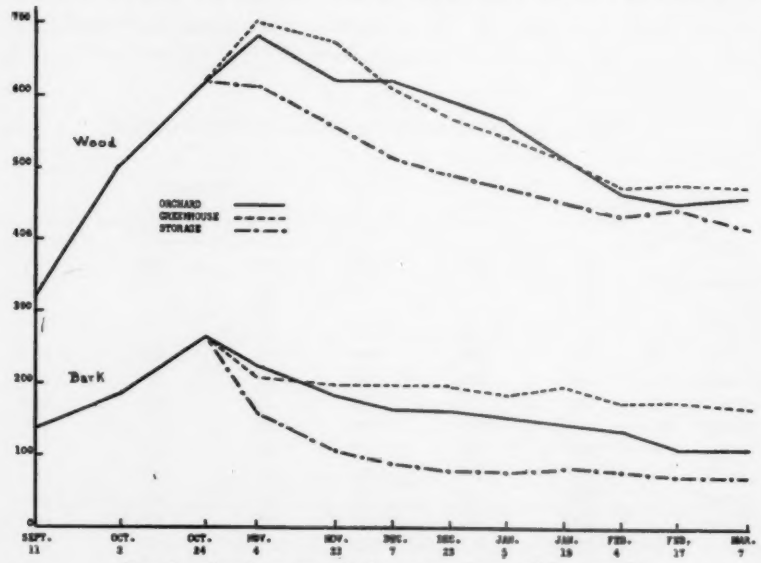

FIG. 6. Starch, as mg. of glucose per 5 gm. of dry sample.

TABLE VIII

WATER-SOLUBLE POLYSACCHARIDES OTHER THAN STARCH, AS MG. OF GLUCOSE PER 5 GM. OF DRY SAMPLE

DATE	BARK		
	ORCHARD	GREENHOUSE	STORAGE
	mg.	*mg.*	*mg.*
September 11	72		
October 2	68		
October 24	68		
November 4	62	87	108
November 23	82	91	80
December 7	77	85	86
December 23	72	85	83
January 5	54	64	68
January 19	69	80	58
February 4	78	68	71
February 17	87	98	77
March 7	72	112	77

probably includes water-soluble hexosans and pentosans among other things. The table is included for the purpose of showing the magnitude of this fraction.

Complete data on the fat content of the shoots was not obtained. However, benzene extractions were made on material collected January 19 from three sets of trees and showed no appreciable difference between the three sets. At the time of this collection, other constituents were showing maximum differences as the result of temperature. In all cases the weight of the benzene extract was very small and appeared to be largely due to pigments.

Total nitrogen (table IX), as might be expected of trees standing in pure sand, remained practically constant. There is perhaps a tendency on the part of the bark of orchard and greenhouse trees to increase in nitrogen, but it will be observed that the wood of these trees decreases in nitrogen about the same extent. Possibly there was a migration of nitrogen from wood to bark at these temperatures, whereas at the low storage temperature no such transfer took place. In any event, temperature apparently does not influence the total nitrogen of the entire shoot, nor is the total amount of nitrogen in itself a factor related to growth renewal.

Little can be concluded regarding the relation of the amide nitrogen fraction to the growth responses of the trees from the three sets (table X). There does, however, appear to be some indication from the data of orchard

TABLE IX

TOTAL NITROGEN, AS MG. PER 5 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
September 11	50.5			30.5		
October 2	51.5			28.6		
October 24	53.3			28.2		
November 4	54.2	52.5	53.3	28.0	29.4	29.4
November 23	53.8	54.8	52.9	26.3	28.4	29.0
December 7	53.3	55.5	52.0	25.3	28.0	29.8
December 23	54.8	55.7	54.3	22.7	26.0	28.4
January 5	56.5	57.8	52.9	22.4	25.8	28.0
January 19	58.1	58.5	53.0	22.2	25.2	28.1
February 4	58.0	60.1	54.0	23.1	25.0	28.4
February 17	60.4	59.5	53.7	23.5	25.2	28.3
March 7	61.2	58.7	54.3	24.4	24.1	29.4

trees that amide nitrogen is relatively high in the fall while the trees are active, is lower during the dormant season, and again increases as growth begins in the spring. The vernal increase, however, did not take place until *after* growth initiation.

The changes in freezing point depression of the expressed sap under the three temperature conditions are shown in table XI and figure 7. The

TABLE X

AMIDE NITROGEN, AS MG. PER 10 GM. OF DRY SAMPLE

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
September 11	6.30			4.55		
October 2	5.03			3.14		
October 24	4.75			2.50		
November 4	4.55	5.23	4.98	2.16	2.32	2.94
November 23	4.75	5.20	4.31	2.27	2.10	2.82
December 7	4.53	4.55	4.33	2.13	2.21	2.97
December 23	4.98	5.03	4.86	1.76	2.12	2.66
January 5	5.06	4.98	4.62	1.76	2.10	2.66
January 19	5.06	5.06	4.84	1.99	2.10	2.50
February 4	4.90	5.06	4.28	1.85	2.10	2.80
February 17	5.03	4.90	4.15	1.88	1.88	2.72
March 7	5.25	4.65	4.55	1.96	2.13	2.82

TABLE XI

FREEZING POINT DEPRESSION OF EXPRESSED SAP

DATE	BARK			WOOD		
	ORCHARD	GREENHOUSE	STORAGE	ORCHARD	GREENHOUSE	STORAGE
	deg. C.	*deg. C.*	*deg. C.*	*deg. C.*	*deg. C.*	*deg. C.*
September 11	1.11			0.58		
October 2	1.25			0.56		
October 24	1.22			0.49		
November 4	1.20	1.22	1.25	0.51	0.54	0.56
November 23	1.21	1.28	1.39	0.55	0.54	0.62
December 7	1.26	1.25	1.58	0.53	0.52	0.70
December 23	1.31	1.28	1.65	0.55	0.62	0.75
January 5	1.43	1.29	1.65	0.58	0.61	0.76
January 19	1.43	1.28	1.59	0.62	0.55	0.75
February 4	1.46	1.24	1.64	0.64	0.57	0.76
February 17	1.39	1.21	1.63	0.59	0.54	0.75
March 7	1.33	1.24	1.71	0.58	0.55	0.80

differences in freezing point depression probably can not be attributed to electrolytes, since the electrical conductivity remains uniform. It is interesting that the freezing point curves follow in general the curves for

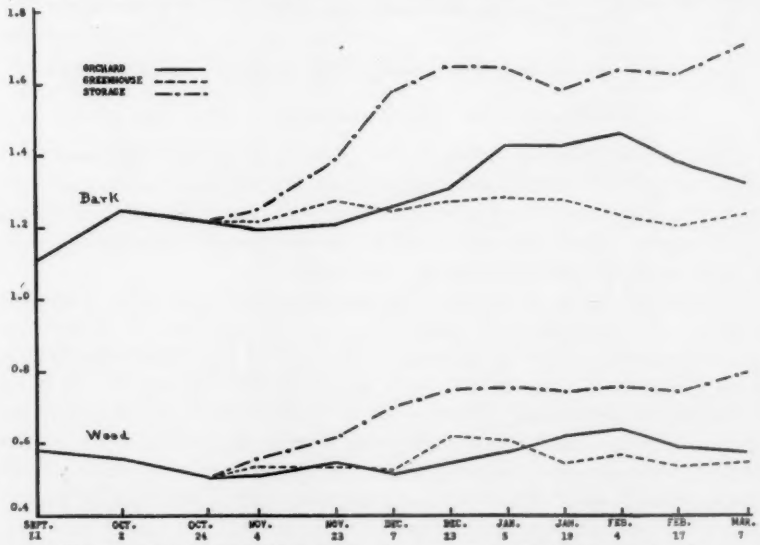

FIG. 7. Freezing point depression of expressed sap, in degrees centigrade.

hexose and sucrose. Although the sugars are the only sap-soluble constituents found thus far to undergo changes comparable to the changes in freezing point depression, it does not appear likely that they could be wholly responsible for such marked freezing point differences. For this reason the depressions which would be contributed by the sugars have been subtracted from the experimental freezing point determinations, and the curves thus redrawn to exclude sugar effects (fig. 8).

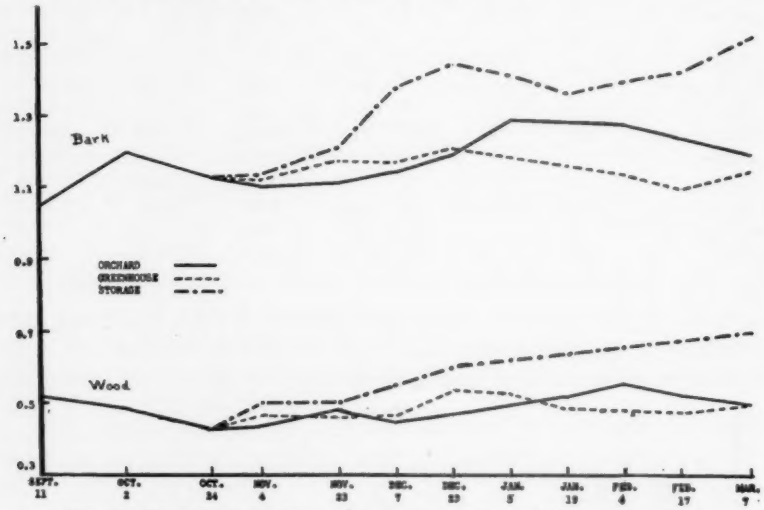

FIG. 8. Freezing point depression of expressed sap minus sugar effects.

The revised freezing point curves make it apparent that some sap-soluble substance or substances not yet accounted for show marked changes under the influence of low temperature, and that they simulate the behavior of the sugars. This observation led to the investigation of organic acids, or their esters, in the expressed sap (table XII).

The organic acids of the sap, or their esters, occur in rather large proportions, equivalent in some instances to a 0.4 N solution. Low temperatures appear to increase the amount of this fraction, the effect being similar to the behavior of the sugars though not so marked (figure 9). It is believed that the remaining difference found in the freezing point depression curves after the deduction of sugars can largely be accounted for by this fraction, although as explained before, this determination of acids is only an approximate one. The results are, however, sufficiently reliable for the conclusion that low temperatures result in appreciable changes in the acid content of the sap, these changes being similar to those which take place in the sugars and in the freezing point depression.

TABLE XII

ORGANIC ACIDS OR THEIR ESTERS, EXPRESSED AS CC. OF 0.1 N ACID PER 10 CC. OF SAP

DATE	BARK		
	ORCHARD	GREENHOUSE	STORAGE
	cc.	*cc.*	*cc.*
November 4	28.3	28.0	30.8
November 23	28.7	29.0	35.0
December 7	29.3	28.5	36.2
December 23	30.3	29.0	38.0
January 5	31.6	29.0	37.7
January 19	30.2	28.4	37.0
February 4	34.2	28.0	39.1
February 17	33.8	27.0	39.0
March 7	33.5	28.4	41.2

Discussion

The Bartlett pear tree, after subjection to sufficient chilling, shows a very definite growth response which does not occur if the temperature remains relatively high during the dormant season. Trees which were moved into the warm greenhouse in October, without previous chilling, were still practically dormant eleven months later, although temperature and moisture conditions were favorable for growth at all times. Exposure to low temperature apparently brings about a change in some internal condition which is necessary for the beginning of growth.

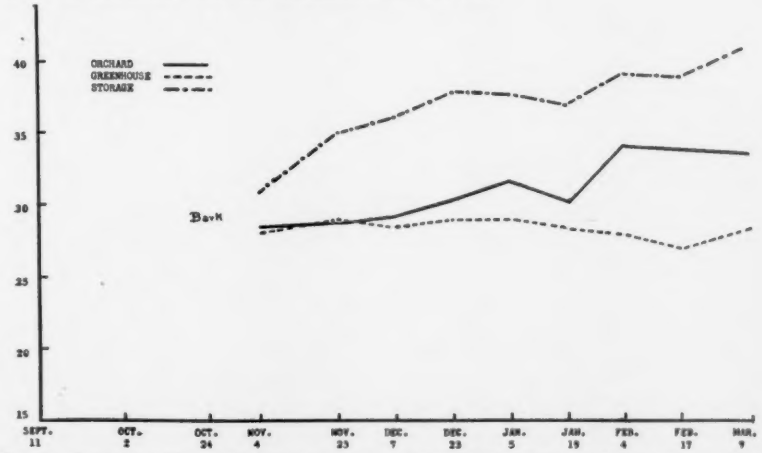

FIG. 9. Organic acids, or their esters, as cc. of 0.1 N NaOH to neutralize 10 cc. of expressed sap.

The changes in composition which are induced by exposure to relatively low temperatures are found to occur chiefly in the carbohydrates. The increase in sugars, although very marked and correlated with the ability of the shoots to grow, has not been established as necessary for the growth response. COVILLE (**6**) believes that a high concentration of sugar initiates the growth of the buds by means of the high osmotic pressure thus produced. APPLEMAN (**2**), on the other hand, considers the increase in sugars, resulting from exposure to low temperature, to be no essential part of the after-ripening process in potato tubers. Although a supply of soluble carbohydrates is necessary for growth, there is no positive evidence that a high accumulation of sugars must occur before growth can begin. However, the greenhouse trees did not accumulate sugars and did not grow.

The starch in all trees was shown to decrease after the fall maximum, but not to the same extent under each temperature condition. The greatest decrease occurred at the lowest temperature. Presumably the starch was converted into sugar, and the accumulation of sugars at low temperature is perhaps the result of a retarded rate of respiration. Although enough starch disappeared from the greenhouse trees to bring about a high concentration of sugar, respiration at the greenhouse temperature may have been so rapid that sugar could not accumulate. In the pear shoot the difference in sugar content between the three sets of material may thus be largely due to a difference in the respiratory rate, although it has been shown that the disappearance of starch is more marked at the lower temperatures.

The secondary starch maximum, reported by FISCHER (**13**), HOOKER (**16**), DU SABLON (**11**), and others, to occur in the early spring, was not found in the pear shoots under any of the temperature conditions. CAMERON (**5**), using microchemical methods, reports this secondary starch maximum in the Bartlett pear to take place in February just before the beginning of growth. A secondary starch maximum in leafless trees would presumably be accompanied by a decrease in sugars or other carbohydrates. Usually the amount of sugar present in the plant is not enough, if all converted into starch, to result in the marked starch regeneration often reported. Whatever the source of the secondary starch maximum, it appears unrelated to the cessation of the rest period, for both storage and outdoor trees exhibited the ability to grow without first showing a second increase in starch.

It was previously mentioned that the increase in fat content, reported by a number of investigators to take place during the winter months, does not appear to be of universal occurrence in all trees subjected to low temperatures. Apparently it does not occur in shoots of the Bartlett pear tree. NIKLEWSKI (**27**) found no supporting evidence of such a change in branches of *Prunus, Betula,* or *Tilia* under different temperature condi-

tions; and POJARKOVA (**32**) has reported an absence of fats in a number of deciduous species. It appears that a change in fat content does not always accompany the ending of the rest period.

In so far as the nitrogenous constituents were examined, the results agree with the findings of APPLEMAN (**2**), MÜLLER-THURGAU (**25**), and DENNY (**9**), that the cessation of the rest period is apparently not dependent upon nitrogen transformations. It is not felt, however, that the incomplete partition of total nitrogen here effected is adequate for a definite conclusion regarding this point. Although no appreciable changes were found in the total and amide nitrogen under the different temperature conditions, the amide fraction as determined constitutes such a small part of the total nitrogen that important changes may have occurred in the other forms of nitrogen which make up the large remainder.

All the constituents of the pear shoot here investigated, with the exception of starch, are much more abundant in the bark than in the wood. That this is true, also, of total soluble organic materials and electrolytes is shown by the freezing point depression and electrical conductivity of expressed sap. Not only is the bark richer in food substances, but the greatest changes under the influence of low temperature occur here. Perhaps this is explainable in that bark has a relatively greater proportion of living cells than wood. It is possible that a finer division of tissues, than the gross separation of bark from wood, should have been made before analysis. HOWARD (**19**) believes that the secret of the rest period resides in the buds themselves rather than in the cambium or any other tissues of the branch. HODGSON (**15**) found that the breaking of the rest by etherization was accompanied by a disappearance of starch from the buds.

By means of chemical stimulation, DENNY and STANTON (**10**) have awakened individual buds on a shoot without influencing the remaining buds. They state that the rest period is not systemic but is localized in the buds and that, therefore, any analyses to elucidate the rest period should not include other tissues. The fact should be recalled, however, that individual buds can also be awakened by injury to the stem. Injury to the bark, such as ringing, notching or bruising, will initiate the growth of buds immediately below the injury although these buds, themselves, are not touched. The writer inclines to the view that the rest period *is* systemic in the sense that it is common to the meristems of the entire plant whether in the bud, stem or root, and that any localized portion of the meristem will renew activity when the conditions either within that portion of meristem or in the tissues surrounding it are favorable for growth.

The theory that enzyme inactivity in the fall is responsible for the beginning of the rest period and is the result of high organic accumulation, and that enzyme reactivation, when that accumulation has been respired

or otherwise removed, initiates growth in the spring, has found favor with a number of writers. In this work, however, there seems to be no evidence that enzymes could be inactivated in the fall by a high organic accumulation, or that reactivation in the spring is resumed because of the removal of that accumulation; for in both the fall and the spring there was found the reverse of the conditions proposed by the theory as regards accumulation of soluble organic materials. In the fall, throughout the period when the pear shoots were presumably entering the rest period, hexose, sucrose, and freezing point depression, which is indicative of the amount of soluble material, were relatively low; while at the time of the cessation of the rest period, the accumulation of soluble organic materials was at a peak. The accumulation of insoluble substances, such as starch, would be less liable to retard enzyme activity.

Since the changes in composition reported in this work were presumably brought about by enzymes, it is interesting, in view of the large temperature coefficients for enzyme action, that the most pronounced changes were found in the trees held constantly at such a low temperature, while the least change occurred in the trees maintained at a high temperature. Although differences in the rate of respiration have probably modified the changes occurring, certain enzyme activity was apparently greater at the low temperatures; for example, the disappearance of starch and also the formation of sucrose were greatest at 2° C. Perhaps, within the plant, the chemical equilibrium points of various reactions which are catalysed by enzymes shift with changes in temperature, or possibly the amounts of enzymes produced at the different temperatures, rather than the activity of a constant amount, may be responsible for the effect. APPLEMAN (**2**), however, reports no difference in the amounts of diastase or invertase activity in chilled and unchilled potato tubers, yet marked changes in the carbohydrates occurred. COVILLE'S concept (**6**), that the enzymes are separated from their substrata by membranes which become more permeable after sufficient chilling, appears to explain the results, although it is without supporting evidence.

It is apparent that further work is necessary in order to explain the effect of low temperature in bringing about the growth of plants. Several other avenues of attack on this problem of the rest period suggest themselves as being of promise. While this work indicates the changes which occur, or fail to occur, in a number of plant constituents, it does not preclude the possibility of important changes in constituents other than those investigated. In addition to a more complete analysis than was made in this work, the changes which occur in the various tissues might be determined. As has been pointed out, it is not known in what tissue or tissues the secret of the rest resides and a gross analysis of bark and wood may

serve to dwarf changes in some constituents which may actually be significant in a particular tissue.

Perhaps some indication as to whether sugars, organic acids, or other substances which increase under low temperature, are essential to the breaking of the rest, may be gained by introducing them singly or in combination into dormant trees. The after-ripening of seeds has apparently been shortened by treatment with sugars (**20**) (**22**) and acids (**12**).

In addition to exposure to low temperatures, other methods which break the rest period—warm baths, etherization, etc.—could be studied in a similar manner to determine their effect on the constituents of the plant. Perhaps certain changes in composition would be found common to all methods of stimulation. From the convergent lines of evidence then available a satisfactory conclusion might be reached regarding what internal conditions of the plant are essential for the renewal of growth.

Summary

1. Dormant Bartlett pear trees were maintained throughout the winter and spring months at different temperatures. Some of the trees were kept in cold storage at a constant temperature of 2° C.; others in the greenhouse where the temperature was never lower than 16° C.; still others at the variable intermediate temperature conditions outdoors.

2. Trees kept in the warm greenhouse remained practically dormant throughout the year, while those exposed to cold storage and to outdoor conditions became capable of growth. The storage trees, which were subjected to a lower temperature than outdoor trees, were first to finish their rest period.

3. At frequent intervals shoots were removed from the trees for analysis in order to ascertain the changes in certain constituents taking place under each of the temperature conditions.

4. The low temperature of the storage and the relatively low outside temperature, both of which resulted in a breaking of the rest period, brought about the following changes in the composition of the shoots: an increase in hexose sugar, sucrose, organic acids, and freezing point depression of the expressed sap, while starch markedly decreased. Little change in these constituents occurred in the trees held in the warm greenhouse.

5. No effect of the different temperature conditions was found in the following determinations: non-hexose reducing substances, water-soluble polysaccharides other than starch, arbutin, fats, total nitrogen, amide nitrogen, and electrical conductivity of expressed sap.

6. The changes reported as the result of exposure to low temperature have not been established as necessary to the initiation of growth; however, growth did not take place in the absence of these changes.

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LITERATURE CITED

1. ABBOTT, O. Chemical changes at beginning and ending of rest period in apple and peach. Bot. Gaz. **76**: 167–184. 1923.
2. APPLEMAN, C. O. Biochemical and physiological study of the rest period in the tubers of *Solanum tuberosum*. Bot. Gaz. **61**: 265–294. 1916.
3. BORESCH, K. Zur Analyse der frühtreibenden Wirkung des Warmbades. Biochem. Zeitschr. **153**: 313–334. 1924.
4. BUTLER, O. A note on the significance of sugar in the tubers of *Solanum tuberosum*. Bull. Torrey Bot. Club **40**: 110–118. 1923.
5. CAMERON, S. H. Storage of starch in the pear and apricot. Amer. Soc. Hort. Sci. **20**: 98–100. 1923.
6. COVILLE, F. V. The influence of cold in stimulating the growth of plants. Jour. Agr. Res. **20**: 151–160. 1920.
7. CROCKER, WM. Rôle of seed coats in delayed germination. Bot. Gaz. **42**: 265–291. 1906.
8. DAVIS, W. E., and ROSE, R. C. The effect of external conditions upon the after-ripening of the seeds of *Crataegus mollis*. Bot. Gaz. **54**: 49–62. 1912.
9. DENNY, F. E. Chemical changes induced in potato tubers by treatments that break the rest period. Amer. Jour. Bot. **16**: 326–337. 1929.
10. ————, and STANTON, E. N. Localization of response of woody tissues to chemical treatments that break the rest period. Amer. Jour. Bot. **15**: 337–344. 1928.
11. DU SABLON, LECLERC. Recherches physiologiques sur les matières de réserves des arbres. Rev. Gen. Bot. **16**: 339–368, 386–401. 1904.
12. ECKERSON, S. A physiological and chemical study of after-ripening. Bot. Gaz. **55**: 286–299. 1913.
13. FISCHER, A. Beiträge zur Physiologie der Holzgewächse. Jahrb. wiss. Bot. **22**: 73–160. 1891.
14. GAIL, F. W. Osmotic pressure of cell sap and its possible relation to winter killing and leaf fall. Bot. Gaz. **81**: 434–445. 1926.
15. HODGSON, F. R. Observations on the rest period of deciduous fruit trees in a mild climate. Proc. Amer. Soc. Hort. Sci. **20**: 151–155. 1923.

16. HOOKER, JR, H. D. Seasonal changes in the chemical composition of apple spurs. Missouri Agr. Exp. Sta. Res. Bull. 40. 1920.
17. HOPKINS, E. F. Relation of low temperatures to respiration and carbohydrate changes in potato tubers. Bot. Gaz. **78**: 311–325. 1924.
18. HORNE, W. J., WELDON, G. P., and BABCOCK, E. B. Resistance of peach hybrids to an obscure disease in southern California. Jour. Hered. **17**: 98–104. 1926.
19. HOWARD, W. L. An experimental study of the rest period in plants: Physiological changes accompanying breaking of the rest period. Missouri Res. Bull. 21. 1–72. 1915.
20. IVES, S. A. Maturation and germination of seeds of *Ilex opaca*. Bot. Gaz. **76**: 60–77. 1923.
21. KLEBS, G. Über das Treiben der einheimischen Bäume, speziell der Buche. Heidelberg. Akad. Wiss., math. naturw. Kl., Abh. **3**: 1–114. 1914.
22. KNUDSON, L. Non-symbiotic germination of orchid seeds. Bot. Gaz. **73**: 1–25. 1922.
23. LEWIS, F. J., and TUTTLE, G. Osmotic properties of some plant cells at low temperatures. Ann. Bot. **34**: 405–416. 1920.
24. LOOMIS, W. E., and EVANS, M. M. Experiments in breaking the rest period of corms and bulbs. Proc. Amer. Soc. Hort. Sci. **25**: 73–79. 1928.
25. MÜLLER-THURGAU, H. Ueber Zuckeranhäufung in Pflanzentheilen in Folge niederer Temperatur. Landw. Jahrb. **11**: 751–828. 1882.
26. ————, and SCHNEIDER-ORELLI, O. Beiträge zur Kenntnis der Lebensvorgänge in ruhenden Pflanzenteilen II. Flora. **104**: 386–446. 1912.
27. NIKLEWSKI, B. Untersuchungen über die Umwandlung einiger stickstoffreier Reservestoffe während der Winterperiode der Bäume. Beihefte Bot. Centralbl. **19** Abt. 1; 68–117. 1905.
28. ONSLOW, M. W. Plant biochemistry. Second ed. p. 162. 1923.
29. PACK, D. A. After-ripening and germination of Juniperus seeds. Bot. Gaz. **71**: 32–60. 1921.
30. ————. Chemistry of after-ripening, germination, and seedling development of Juniper seeds. Bot. Gaz. **72**: 139–150. 1921.
31. PERETOLCIN, K. Die Änderungen von Reservestoffen bei unseren Bäumen in der Winterruheperiode. Mitteil. des Petersburg. Forst.-Inst. **11**: 3–42. 1904.
32. POJARKOVA, A. Winterruhe, Reservestoffe und Kälteresistenz bei Holzpflanzen. Ber. deutsch. bot. Ges. **42**: 420–429. 1924.

33. Rippel, A. Untersuchungen über die Mobilisation der Aschenbestandteile und des Stickstoffs in Zweigen beim frühjahrlichen Austreiben. Biochem. Zeitschr. **113**: 125–144. 1921.
34. Rosa, J. T. Relation of tuber maturity and of storage factors to potato dormancy. Hilgardia **3**: 99–124. 1928.
35. Shaffer, P. A., and Hartmann, A. F. The iodometric determination of copper and its use in sugar analysis. Jour. Biol. Chem. **45**: 349–390. 1920.
36. Shull, C. A. The oxygen minimum and the germination of Xanthium seeds. Bot. Gaz. **52**: 453–477. 1911.
37. Tuttle, G. M. Induced changes in reserve materials in evergreen herbaceous leaves. Ann. Bot. **33**: 201–210. 1919.
38. Weber, F. Untersuchungen über die Wandlungen des Stärke- und Fettegehaltes der Pflanzen, insbesondere der Bäume. Sitzungsber. Kais. Akad. Wiss. Wien. **118**: 967–1032. 1909.
39. ————. Studien über die Ruheperiode der Holzgewächse. Sitzungsber. Kais. Akad. Wiss. Wien. **127**: 57–91. 1928.

RELATION BETWEEN THE DEVELOPMENT OF ROOT SYSTEM AND SHOOT UNDER LONG- AND SHORT-DAY ILLUMINATION

J. E. Weaver and W. J. Himmel

(with seven figures)

Relatively few investigations have been made to determine the factors which affect the relation of the growth of roots to tops. A more exact knowledge of the relations between aerial and subterranean plant parts and the degree to which these may be modified through cultural practices is of great scientific and practical importance. Extensive investigations have shown that plants exhibit marked specific and varietal differences with respect to relative development of roots when grown under the same environmental conditions. Intensive studies on the growth of wheat by Weaver, Kramer, and Reed (**20**) and of certain other plants by Crist and Stout (**3**) have also made clear that there is a persistent tendency towards a positive correlation between roots and shoots, increase in size of tops being accompanied by increase in size of roots. Although significant variations in the relative distribution of the growth rate of tops and roots occur in nature and may readily be induced by change in environment, the fact remains that there is a persistent positive correlation in size of tops and roots regardless of the wide variations induced by special conditions.

The maintenance of a proper balance between root and shoot is of very great importance. If either is too limited or too great in extent, the other will not thrive. The root system must be sufficiently widespread to absorb enough water and nutrients for the stem and leaves, which, in turn, must manufacture sufficient food for the maintenance of the root system. The plant is a biological unit although it is frequently not treated as such. It is often mutilated by pruning, cutting or injuring the root system, frequently without much regard to the effect upon the remaining portion.

Jean and Weaver (**7**), Harris (**6**), and others have shown that the general effect of decreased water content of soil, providing enough is available to promote moderate growth, is to accelerate root development in relation to growth of tops. Low humidity resulting in high transpiration gives similar effects. Maximow and Lebedincev (**13**), Maximow *et al* (**14**), and Turner (**18**), have found that plants grown in shade have a less extensive root system and a greater ratio of dry weight of tops to roots. Moeller (**15**), Gericke (**5**), and Livingston (**9**) have shown that in sterile soils or in dilute nutrient solutions, roots develop extensively in relation to

tops and the ratio of dry weight of tops to roots is greatly decreased. Conversely, a generally enriched soil or one with an abundance of nitrates increases the ratio of tops to roots (**6, 18, 3**). CHANDLER (**2**), for example, found that "while the nitrate has increased both top and root growth, it has not increased root growth to as large an extent as it has increased top growth. Thus, while the top growth of fertilized trees [peaches] is twice as great as that of the unfertilized trees, the root growth is only 50 per cent. greater." As pointed out by TURNER (**18**), the decreased root growth in the presence of an abundance of nitrogen may result from a low supply of carbohydrates. As regards the roots of seedlings, it has been shown that their development is greatly influenced by the nature of the reserve food supply in the seed. The more nitrogen a seed contains, the greater is its shoot growth as compared to roots. REID (**17**) has shown that an abundance of carbohydrate foods and a somewhat limited nitrogen supply promote rapid root development.

Cultural practices such as pruning the tops, etc., may affect the normal correlation between the development of root and stem in a very definite manner. AUSTIN (**1**), for example, reports experiments with 3-year-old almond trees where the development of both the tops and root systems was inversely proportional to the severity of the pruning of tops. The spread of the roots was over one-third greater where the pruning was light than where it was severe.

The preceding data are sufficient to indicate that a plant is a very plastic organism and that any factor that influences the development of either root or shoot may also profoundly affect the other. The extensive researches of GARNER and ALLARD, which have since been supplemented by those of several other investigators, show clearly the profound effect that the period of daily illumination exerts upon the vegetative and reproductive activities of plants. However, so far as the writers are aware, relatively little attention has been given to the effect upon the root systems. GARNER and ALLARD (**4**) state that "preliminary observations indicate that the duration of the daily illumination period may exert a marked effect on the relative development of the root and the aerial portions of the plant. For example, a cutting of Biloxi soybeans made no top growth at all through the winter months and the original leaves assumed a very dark color and generally unthrifty appearance. Apparently new buds were unable to develop. Upon examination of the underground portion of the plant in the spring it was seen that the soil contained a large mass of roots altogether out of proportion to the top of the plant, as judged by the usual summer growth. Other similar cases have been observed in which a light duration unfavorable to aerial development has caused extensive root growth.

Growth of root and shoot, therefore, are not necessarily contemporaneous with respect to season, and arrested development of the exposed portion of the plant caused by suboptimal light duration need not be accompanied by checking of root growth."

Under periods of illumination varying from 5 to 24 hours duration, PFEIFFER (**16**) found that the amount of development in the fibrous root systems of tomato and buckwheat appeared roughly comparable with that of the aerial parts of the same plants.

JOHANSSON (**8**) showed that root development, in proportion to the total weight of the plant, increased with an increase in light intensity. For intensities of 39 and 70 per cent. there is a falling off in the rate of increase, in proportion to total weight, at 10 or more hours daily illumination and for an intensity of 23 per cent. a decrease begins at 12 hours daily illumination.

LUBIMENKO and SZEGLOVA (**12**) found that the weight of the roots increased in proportion to the weight of the whole plant as the length of the daylight periods became progressively longer.

CRIST and STOUT (**3**) grew plants in 6-inch pots at East Lansing, Michigan. They found in the case of lettuce and radish that shortened periods of illumination (6 hours daily) gave a greater ratio of tops to roots, based on dry weight, than either the normal length of day in cloudy weather of winter or one prolonged 6 hours by means of artificial illumination. Moreover, the plants grown under the longest period of illumination had the lowest top-root ratios while the short-day plants had the lowest actual weight of both tops and roots.

Undoubtedly the most significant criterion for judging the efficiency of root development is measurement of the actual absorbing surface. Because of the great difficulties of procedure this has been accomplished only in a few instances (**20, 19**). The method of determining dry weights has a twofold handicap. Where roots are grown in the soil it is practically impossible to free them completely from adhering rock particles without losing some of the roots. But a much more serious objection is encountered in the fact that this method when applied to maturing plants gives little idea of root efficiency. The woody tissues of a single main root that had ceased functioning often far outweighs an extensive network of actively absorbing rootlets. Consequently, it was deemed best in these experiments to use containers sufficiently large to hold a mass of soil in which the roots could develop in a somewhat natural manner. By accurately measuring and comparing root lengths and diameters, and the number, size, and degree of branching, a close approximation to the relative root development of plants grown under different conditions was obtained. To check out variations within the individual, several plants of each species were grown.

Procedure

In these experiments, the following plants were employed: red clover (*Trifolium pratense*), white icicle radish (*Raphanus sativus*), an iris (*Iris germanica*), common sunflower (*Helianthus annuus*), yellow duke dahlia (*Dahlia pinnata*), great ragweed (*Ambrosia trifida*), white Kherson oats (*Avena sativa*), and an early flowering cosmos (*Cosmos bipinnatus*). These were grown in heavy galvanized containers, 30 cm. square and 60 cm. deep, into the top of each of which was inserted a tight wooden frame in such a manner that the soil depth was increased 20 cm. The crevices were luted with plasticine. The containers were filled with a rich loam soil of optimum and uniform water content, which was well screened and exceptionally free from partially decayed roots or other debris. The water-holding capacity of the soil was about 50 per cent. (Hilgard method) and the hygroscopic coefficient 8.2. In filling the containers, the soil was moderately compacted, and the original water content was maintained by adding water from time to time in sufficient amounts to restore the original weight. Two containers were used for each species.

The ragweeds were transplanted from the field in the early seedling stage without injury to the root system. Iris was propagated by rhizomes 1 to 1.5 inches long, carefully selected for uniformity in size, the leafy tops being cut back to within 4 inches of the rhizome. The dahlias were grown from uniformly selected fleshy roots, and all of the other species from seed. After sowing or transplanting, the soil was covered with a fine gravel mulch to retard surface evaporation. The dahlias were planted on May 3, cosmos and clover on May 5, iris on May 8, and the others on May 16. On May 20, one container of each species was placed on an improvised truck so arranged that they could be placed alongside of the other containers from 9:00 A. M. until 4:00 P. M. each day and kept in an adjacent, well ventilated dark room the rest of the time. Thus, one-half of the plants were subjected to a short day of 7 hours' duration, and the rest to the normal 13- to 15-hour day during May and June.

Results

Red clover

The 20 clover seedlings in each container had developed only a single leaf per plant when the difference in length of day was imposed upon them. Within a week the long-day plants had twice the stature of those of the short-day and thereafter differentiation was marked. On June 19, when the containers were opened and the roots excavated, the long-day plants were 28.4 cm. high and in full bloom but the paler colored short-day plants were only 9.8 cm. tall (fig. 1). Leaflets of the short-day plants averaged 1.75 by 2 cm.; those of the long-day 2.5 by 3.5 cm. The larger plants had

FIG. 1. Long-day and short-day plants of red clover 7-weeks old. The long-day plants are in blossom.

25 leaves each, the smaller ones only 12. The result of this difference in photosynthetic surface was clearly shown in the dry weight of tops which was 34.1 and 4.1 grams for the two lots respectively.

Red clover develops a strong taproot from which major laterals arise mostly in the surface 15 cm. of soil. These extend outward more or less horizontally or obliquely and then turn downward and penetrate deeply. At greater depths the primary laterals are smaller and do not spread widely.

The taproots on the short-day plants were only 1.5 mm. thick as compared with 4 mm. for those on the long-day plants. All but one, which barely reached the bottom of the container, ended at 62 cm. or above, while all those of the larger plants extended to and ran along the bottom of the container. The number of major laterals on the proximal 15 cm. of taproot was 3 to 5 per plant, and the total number of laterals was about 40 in both cases. On the short-day plants, however, very few of these extended below the 25 cm. level, a maximum depth of 45 cm. being determined. But those of the other group were as large as the taproots of the smaller plants and most of them extended much deeper, often running along the bottom of the container. The long-day plants alone had root branches of the third order. Below 15 cm. depth, laterals on the short-day taproots were mostly 1 cm. or less in length while both the main roots and primary

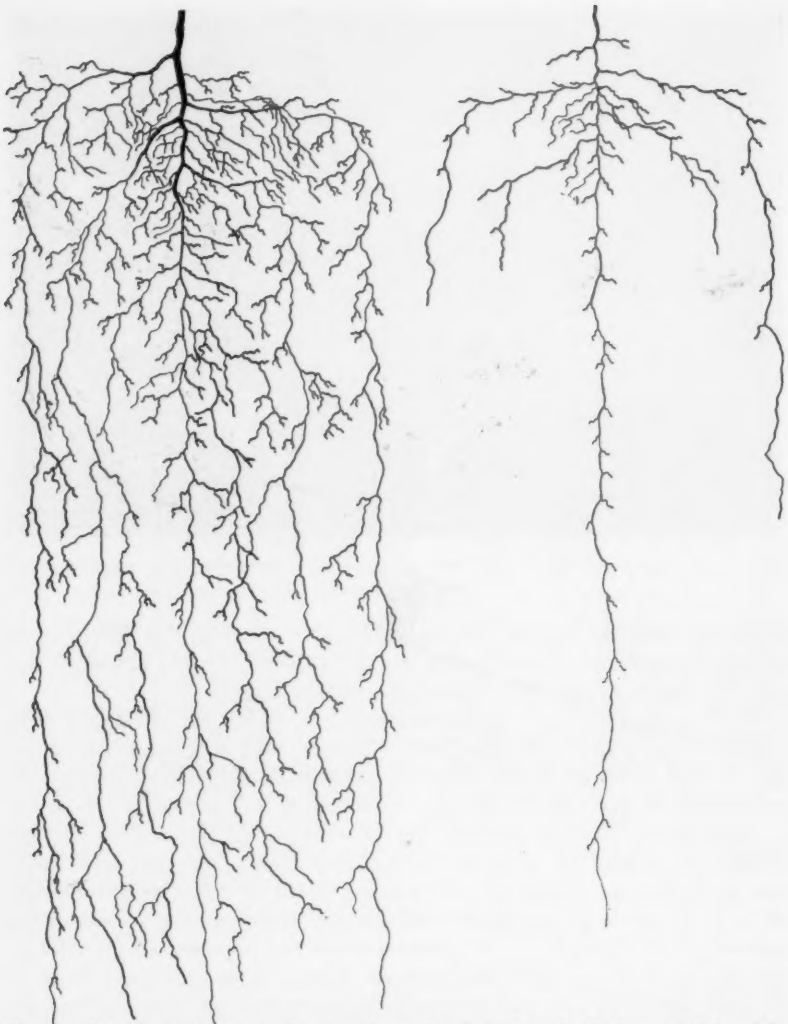

FIG. 2. Relative development of root systems of red clover under 15-hours (left) and 7-hours daily illumination.

branches of the long-day plants had developed many rootlets 5 to 10 cm. long and some reached a length of 36 cm. and extended to the bottom of the container. In fact, the surface 15 cm. of soil of the short-day container was much more poorly ramified with roots than the last 15 cm. of the other

container. Thus while the general plan of the root systems was the same in both cases, roots of the short-day plants, where growth of tops had been inhibited, represented a much earlier stage in development than those of the well developed plants in blossom (fig. 2).

Radish

The radishes were in the cotyledon stage when placed under the two conditions. Within a single week the larger leaves and darker green color of the long-day plants were very noticeable and when the roots were examined at the end of a month marked differences were apparent (fig. 3). Although each of the ten plants possessed the same number of leaves, those of the long-day averaged just three times as large (1,365 sq. cm.) as those of the short-day plants.

Fig. 3. Average development of tops and fleshy portion of roots of long-day (right) and short-day plants of radish.

The radish is characterized by a rapidly growing and deeply penetrating taproot. Strong, profusely branched laterals arise in the surface 7.5 to 20 cm. of soil and constitute the major portion of the absorbing system, since at greater depths the taproot develops only short laterals.

The taproots of the smaller plants (fig. 3) were 20 to 75 cm. long, those of the larger ones reached greater depths and extended 10 to 15 cm. along the bottom of the container. The thread-like lateral roots and their almost cobwebby network of branches were of nearly equal abundance in both containers in the first 20 cm. of soil. But in the next 25-cm. layer this network of branches continued in the long-day plants while in the others it was absent and there were few branches except very close to the taproot. At 40 cm. depth, branches from the taproots averaged somewhat

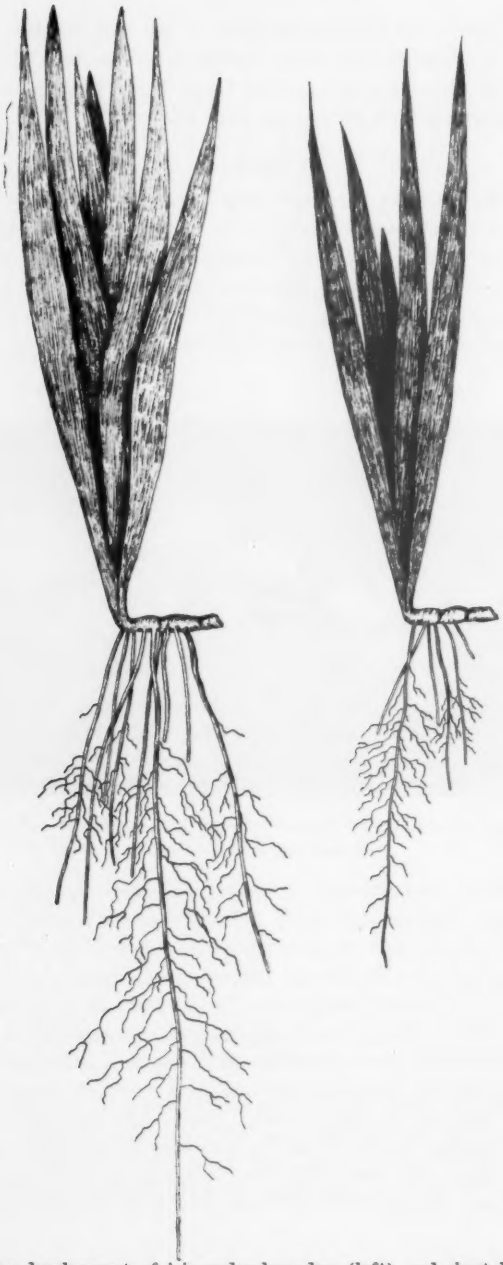

FIG. 4. Relative development of iris under long-day (left) and short-day illumination.

more than twice as long on the larger plants. Even the last 30-cm. layer of soil was penetrated at distant intervals by the vertical ends of these thread-like surface roots.

The fleshy portion of the short-day root system averaged 6 cm. long and 1.7 cm. in diameter; its volume was 58 cc. That of the larger plants was 12 cm. long and 2.7 cm. thick, and the volume 223 cc. Thus the root system of the long-day plants was fully twice as well developed as that of the short-day plants.

Iris

The five rhizomes of iris placed in each of the two containers showed little differentiation of tops until near the time of root excavation on June 19. The average length of leaves of the long-day plants scarcely exceeded that of the short-day but the new leaves were 18 per cent. more abundant. They also averaged about 7 cm. longer and 0.4 cm. wider. The total dry weight of leaves was 2.7 times as great in the long-day plants.

The root system of the iris was very simple. It consisted merely of a few large roots which penetrated rather vertically downward and branched but sparingly. The length and diameter of these roots were carefully measured as they were excavated and counts of the number and measurements of the lengths of the branches were made. On the short-day plants the average number of main roots per rhizome was 6; on the long-day plants 9.2. The former were 1 to 2 mm. in diameter, the latter 1.5 to 3. The average lengths were 13.1 and 22.3 cm. respectively, and the maximum depth of penetration, in the same sequence, 30 and 58 cm. In the short-day container, roots were sparse below 20 cm.; the soil was well filled with roots of the long-day plants to 32 cm. in depth. The younger and shorter roots were unbranched. Primary laterals became more abundant and developed longer branches as the main roots grew longer. Hence, this portion of the absorbing system was much more fully developed in the long-day plants. For example, at 28 cm. depth short-day plants gave off branches only 1 to 4 cm. long, while 12 cm. deeper the larger plants had rootlets 4 to 9 cm. in length which were occasionally rebranched (fig. 4).

Sunflower

Under the long-day illumination the sunflower rapidly developed from the cotyledon stage. The short-day plants were yellowish green and distinctly retarded in their development. They were not attenuated. When the roots were examined on June 17, the long-day plants had a height of 74 cm. and a stem diameter of 15 mm. Each possessed 16 leaves and the average leaf area was 1,924 sq. cm. (fig. 5). The smaller plants were 19 cm. tall and the stems 5 mm. thick. There were only 11 leaves per plant

and the average photosynthetic area was only 14 per cent. as great (270 sq. cm.).

The sunflower has a pronounced taproot with numerous strong lateral branches originating almost entirely in the first few inches of soil. The taproots of the short-day plants were 2 mm. in diameter at a depth of 4 cm.; those of the long-day plants were 12 mm. in diameter. The former

FIG. 5. Long-day and short-day plants of dahlia and sunflower about 6 weeks old. The larger plants grew under a long-day illumination. The containers are 80 cm. deep.

tapered to a width of only 1 mm. at 15 cm. depth but extended to the bottom of the container. Those of the long-day plants were 2 mm. thick at the 15 cm. level and 1 mm. in diameter where they grew along the bottom of the container. The largest branches of the short-day plants were 1 mm. or less in diameter and reached a depth of only 28 cm. Those of the long-day plants were 3 mm. thick, they were much more abundant, and some reached the bottom of the container which was covered with a fairly dense network of roots consisting of taproots and the long surface laterals, and the branches of both lots.

The working level of the primary branches (*i.e.* the depth to which most of the roots extended and to which depth maximum absorption occurred) was 15 cm. in the short-day plants, but 45 cm. in the others. The great mass of roots at the working level of the long-day plants, together with their greater diameters and longer and thicker branches, was in marked contrast to that of the short-day plants. The lateral roots originating below 20 cm. depth were 12 cm. or less in length in the smaller plants; on the larger ones, the secondary rootlets on the bottom of the container were of similar length and development. Below the working level, lateral roots were sparse in the short-day container; there was practically none beyond 55 cm. depth. In the long-day container they were more abundant and of larger diameter than were those of the short-day plants between 15 and 55 cm. depth. Even the last 15 cm. of soil contained numerous rootlets.

Thus the root development of the short-day sunflowers corresponded in every way with that of very much younger plants than those of the long-day which had made a normal development.

Dahlia

The dahlias were about 5 inches tall and were developing a second pair of leaves when the one lot was subjected to the short-day. The two plants of each culture developed leaves at about the same rate but by the end of 15 days the long-day plants were twice as tall and the leaves of a darker green color. When taken off on June 17, the average length of stems was 71 and 26 cm. respectively and the diameter of the stems was almost twice as great in the taller plants. The larger plants had begun to branch and now had more and much larger leaves. The average relative leaf areas were 2,310 and 885 sq. cm. respectively (fig. 5).

The root system of the dahlia consisted of a number of main fibrous roots that arose from the base of the tuberous root. Some of these ran outward and upward, branching profusely to near the soil surface; and still others ran vertically downward. In addition, a few fibrous roots arose from the top of the tuberous root.

The number of roots arising from the base of the old, fleshy root was the same in both lots, varying individually from 10 to 39. They were better developed in the long-day plants where the largest roots were twice as thick, and the branches more numerous, larger, and longer. With a single exception, no roots occurred below 60 cm. on the short-day plants and the working level was 23 cm. The long-day plants extended their roots in considerable numbers quite to the bottom and sometimes along the floor of the container. The working level was nearly three times as great as that of the short-day lot and roots were much more abundant even in the deepest soil than below the 23 cm. level of the short-day plants. More-

over, the network of roots that extended above the bases of the tuberous roots was very much more pronounced in the soil of the long-day plants. Here also this network was found to continue to the working level at about 60 cm.

A lot of tuberous roots had developed from the base of the stems above the original propagule together with a few much-branched fibrous roots. The average number (35) was only slightly greater in the long-day plants where the fleshy roots were long and spindling (8.7 x 0.5 cm.) than in those of the short-day which were short and thick (5.4 x 1.6 cm.). The total volume of the latter (68 cc.) was over 2.5 times that of the long-day plants. While nearly all of the thickened but spindling roots ended without branching, the fleshy root continuations of the short, thick ones were branched, the laterals penetrating widely, and often to the working level.

ZIMMERMAN and HITCHCOCK (**21**) have shown that in certain varieties of dahlia, length of day determines the type of root system formed by cuttings. Heavy root storage is correlated with a short day and a fibrous root system with a long one.

RAGWEED

The ragweeds had a second pair of leaves at the beginning of the experiment. Within a week the long-day plants were twice as tall as those of the short-day, and, as in all other cases, the latter lacked the deep, dark-green color of the long-day lot. By June 27 when the experiment was concluded, the four long-day plants had made a vigorous vegetative growth and attained a height of 90 cm. but showed no signs of flowering. Those of the short-day were only 12 cm. tall and small inflorescences were beginning to appear. Average stem diameters were 10.6 and 4.1 mm. The leaves had three times the length and breadth in the larger plants and the dry weight of tops was 7.6 times greater in the long-day plants (fig. 6).

The root systems of the ragweeds showed almost as marked differences as the tops, those of the short-day plants being very poorly developed. The strong taproots of the larger plants were about 13 mm. in diameter; those of the smaller ones did not exceed 3.5 mm. Although the thread-like taproots of the latter extended to the bottom of the container and often ran 5 to 10 cm. along the bottom, they were poorly developed in comparison to the vigorous taproots of the larger plants, which undoubtedly would have extended two or three feet deeper.

Like the sunflower, this species gives rise to strong laterals in the surface 15 cm. of soil. In fact, these are so vigorous that they often equal the taproot in size and degree of branching. On the long-day plants they were frequently 4 mm. in diameter at their origin and many of them extended along the bottom of the container. In fact, they were larger throughout

FIG. 6. Development of great ragweed at the end of six weeks. The tall plants have had daily about 15 hours of light.

than the taproots of the short-day plants. Major branches of the short-day plants were only one-fourth as large, being mere threads which did not extend beyond 40 to 60 cm. in depth. Laterals, however, were fairly profuse but mostly short and only occasionally of the third order. Laterals on the major branches of the long-day plants were very much more abundant, several times as long, and often rebranched to the fourth order. Even in the center of the container, the secondary branches were as large as any of the primary branches of the taproots of the short-day plants. Smaller branches from the two lots of taproots varied in about the same proportion as those described.

Only in the surface 15 cm. of soil in the short-day containers were roots fairly numerous; at greater depths they were sparse, consisting of the thread-like taproots and their short, scattered branches. Conversely, al-

most every cubic centimeter of the entire soil of the long-day container was thoroughly ramified with roots, those in the upper half being especially dense, although great mats of roots also occupied the bottom layers of soil.

Oats

Because of an accident to one of the containers, it was necessary to replant the oats on June 3. After it was well established, the plants were thinned to 35 per container. Final measurements were made on July 19. The short-day plants were 32 cm. tall and in the fifth-leaf stage, the long-day plants were 53 cm. high, each had 6 or 7 leaves and on some the panicles were beginning to appear. The dry weight of tops was 4.4 and 11.4 grams respectively.

Oats develops a fine, fibrous root system. The primary root system consists (usually) of the three seminal roots (*i.e.* the primary root and two almost equally large laterals) and their branches. The secondary root system, which develops later and constitutes the larger part of the root mass, consists of numerous adventitious roots that develop from the lower nodes of the stem.

Among the short-day plants only a few roots of the primary system extended to the bottom of the container but practically all of those of the long-day not only reached this depth but also ran 10 to 15 cm. along the floor of the container. The latter were also much more profusely furnished with longer branches. Roots of the secondary system did not penetrate beyond 13 cm. in the short-day container, but they reached twice this depth on the long-day plants. Moreover, actual count showed that they were about 4 times as numerous and their branches were longer and well clothed with laterals. Thus the roots of the short-day oats corresponded to a much earlier stage of development than that shown by the long-day plants.

Cosmos

One lot of cosmos was placed under short-day illumination upon the appearance of the first leaves. Paler color and retarded growth were clearly evident a week later and by June 27 when the short-day plants were in full bloom, differences in vegetative growth were marked (fig. 7). The seven short-day plants averaged 35 cm. tall, with a stem diameter of only 2.5 mm. The other seven were 50 cm. high and the stems were 6 mm. thick. The dry weight of the stems, in the same sequence, was 1.4 and 10.0 grams, and that of the leaves 0.6 and 9.6 grams.

Cosmos has a taproot which penetrates deeply, and develops numerous strong laterals in the surface soil. The deeper portion of the taproot is well branched but the branches are relatively short.

FIG. 7. Cosmos about 60 days old. Those receiving daily light for only 6 hours are in bloom. Two of the 7 plants from each container were cut and placed in the jars for photographing.

The main roots of the larger plants were 3 mm. in diameter, and except for the limited depth of soil would probably have extended to depths of 1.25 meters or more. The fine taproots of the smaller plants were no larger near their origin, than those of the larger ones at 80 cm. in depth. They were scarcely 75 cm. long. On the short-day plants 10 to 12 laterals barely 1 mm. thick arose in the surface 10 cm. of soil. The longest extended only 24 cm. and most of them were much shorter. Many of the main laterals were unbranched, others had branches a cm. or less in length. The long-day plants had approximately three times as many strong laterals, some originating at 15 cm. depth. These had twice the diameter and three times the number of laterals as those of the short-day. Moreover, the laterals were 1 to 5 cm. long and frequently rebranched. Branches from the taproot were sparse and short below 35 cm. depth on the short-day plants.

But on the larger plants even at 50 cm. depth they were fairly abundant and some were 10 cm. long. The larger taproots branched throughout their length. The short, fine taproots of the smaller plants, the smaller number and limited extent of the branches, and the smaller number of branch orders all indicated an arrested development which corresponded in general with the lesser development of the tops.

Carbohydrate determinations

The preceding data show that in every case the development of the absorbing system was clearly correlated with the transpiring surface. This was true for the long-day plants, *viz.*, clover, iris, and oats, which require long days for successful flowering and fruiting, as well as for cosmos, ragweed, and dahlia which come into blossom normally only when short days occur. The sunflower, which is not materially influenced in time of flowering by the length of day, at least in medium-high latitudes, likewise showed a clear correlation between development of root and shoot.

Where, as in this experiment, all conditions except that of length of day are favorable for growth, it would seem that development of both tops and roots would be directly correlated with the rate of food manufacture. This is clearly indicated by a series of measurements on the rate of carbohydrate manufacture in several of the species.

In these experiments, the photosynthetic activity is based on total carbohydrate content and upon increase in carbohydrate content of leaves after insolation, this being measured as reducing sugars. The photosynthetic activity was determined by the picric acid colorimetric method as modified by Long (**10, 11**).[1]

Photosynthetic determinations were made on the leaves of sunflower, radish, and dahlia on June 13 and 15; of the ragweed on June 13, 15, and 26; and of the red clover on June 26 and 27. All except June 27 were bright sunny days. Samples of the plants growing under long-day exposure were secured at daylight, 9:00 A. M., 4:00 P. M., and sunset. Those from plants under the short-day were taken at 9:00 A. M. and 4:00 P. M. In all instances, care was taken to secure samples from fully grown leaves of approximately the same age, that were shaded as little as possible.

Table I gives the actual amount of sugar per square centimeter in each sample, the relative amounts in the long- and short-day plants at 4:00 P. M., the increase in sugar content during the entire period of illumination, and the actual and relative increases between 9:00 A. M. and 4:00 P. M. for both sets of plants.

[1] These data are from unpublished work by Miss Theodora Klose, to whom a detailed outline of the method was furnished by Doctor Long.

TABLE I

PHOTOSYNTHETIC ACTIVITY OF LONG- AND SHORT-DAY PLANTS

			Sunflower				Radish				Clover			
			June 13		June 15		June 13		June 15		June 26		June 27	
			Actual	Relative	Actual	Relative	Actual	Relative	Actual	Relative	Actual	Relative	Actual	Relative
			mg.	*per cent.*	*mg.*	*per cent.*	*mg.*	*per cent.*	*mg.*	*per cent.*	*mg.*	*per cent.*	*mg.*	*per cent.*
Sugar per sq. cm.	Long day	Daylight	0.704		0.640		0.704		0.640		0.768		0.640	
		9 A. M.	0.832		0.640		0.704		0.640		0.768		0.768	
		4 P. M.	1.152	100	1.088	100	0.896	100	1.024	100	2.368	100	1.408	100
		Sunset	0.896		0.960		0.896		0.960		1.024		1.024	
	Short day	9 A. M.	0.640		0.640		0.704		0.640		0.384		0.423	
		4 P. M.	0.768	67	0.896	82	0.768	86	0.960	94	0.872	37	0.646	46
Increase per sq. cm.	During entire day	Long day	0.448		0.448		0.192		0.384		1.600		0.768	
		Short day	0.128		0.256		0.064		0.320		0.488		0.223	
	Between 9 A. M.–4 P. M.	Long day	0.320	100	0.448	100	0.192	100	0.384	100	1.600	100	0.640	100
		Short day	0.128	40	0.256	57	0.064	33	0.320	83	0.488	31	0.223	35

TABLE I.—(*Concluded*)

			Ragweed						Dahlia			
			June 13		June 15		June 26		June 13		June 15	
			Actual	Relative	Actual	Relative	Actual	Relative	Actual	Relative	Actual	Relative
			mg.	*per cent.*	*mg.*	*per cent.*	*mg.*	*per cent.*	*mg.*	*per cent.*	*mg.*	*per cent.*
Sugar per sq. cm.	Long day	Daylight	0.768		0.640		0.512		1.024		0.768	
		9 A. M.	0.640		0.640		0.704		0.896		0.768	
		4 P. M.	1.024	100	1.088	100	0.992	100	1.152	100	1.280	100
		Sunset	1.088		1.024		0.896		1.024		1.280	
	Short day	9 A. M.	0.704		0.640		0.576		0.768		0.640	
		4 P. M.	1.152	113	1.152	106	0.896	90	1.024	89	1.152	90
Increase per sq. cm.	During entire day	Long day	0.448		0.448		0.480		0.256		0.512	
		Short day	0.448		0.512		0.320		0.256		0.512	
	Between 9 A. M.–4 P. M.	Long day	0.384	100	0.448	100	0.288	100	0.256	100	0.512	100
		Short day	0.448	117	0.512	114	0.320	111	0.256	100	0.512	100

An examination of the table shows that on both June 13 and 15 the daily rate of increase in sugar in the sunflower was considerably higher in the long-day plants, and also that greater amounts of sugar per unit area occurred in the plants having the longer illumination.

Photosynthetic tests of the radish show, as in the sunflower, a much higher rate of daily increase in sugar content and also larger amounts of sugar per sq. cm. The differences are somewhat less on June 15, probably owing to differences in the place of selecting samples or in environment, but the decrease is consistent in the two species.

The red clover gave not only the greatest increase in photosynthate of any of the plants tested but also the greatest differences between the long- and short-day plants. Table I shows a much higher rate of increase in sugar content between 9:00 A. M. and 4:00 P. M., as well as during the entire period of illumination of the two lots of plants. The smaller amounts of photosynthate on June 27 are due to the fact that this was a cloudy day.

In contrast to the behavior of the three preceding species, ragweed and dahlia under long daily illumination gave no higher rates and, in fact, often lower ones, than those under 7-hour daily periods of light. The photosynthetic tests on the ragweed on June 13 showed a slightly higher rate of increase in the short-day plants between 9:00 A. M. and 4:00 P. M. The total daily increase during the entire period of illumination is, however, the same. The actual amounts of sugar at 4:00 P. M. are greater in the short-day plants than in those of the long-day. The tests on June 15 are essentially the same except that the total daily increase is greater in the short-day plants instead of being equal in the two sets as on June 13. On June 26 the rate of increase between 9:00 A. M. and 4:00 P. M. still remained higher in the short-day than in the long-day plants; contrary to the previous results, however, the total increase during the entire period of illumination of the long-day plants was greater than the increase during the 7-hour period of illumination of the short-day plants. The actual amount of sugar at 4:00 P. M., moreover, was greater in the former. The short-day plants were beginning to flower which, no doubt, resulted in rapid use of the photosynthate and perhaps accounts for the lower actual amount of sugar in these plants at this time. The rate of increase remained higher throughout in the short-day plants.

As regards the dahlia, table I shows a slightly higher amount of sugar at 4:00 P. M. in the leaves of the long-day plants than in those of the short-day. The rate of increase, however, between 9:00 A. M. and 4:00 P. M. is the same in the two sets of plants. The increase during the 7-hour period of light of the short-day is equal to the increase of the long-day plants during their entire period of illumination.

In interpreting these results it must be kept in mind that the rate of increase in these tests represents the balance between the process of photosynthesis on the one hand and those of respiration, translocation, and growth on the other. Hence, the higher rate obtained under certain conditions is not necessarily due to a higher rate of manufacture of materials under these conditions but may result in part from a slower rate of respiration, or removal, or growth.

GARNER and ALLARD have found that there are different optimal light periods for maximum vegetative growth, for sexual reproduction, and for tuberization, each of which varies with the species. The photosynthetic data given above seem to indicate that those plants, such as the radish and clover, whose optimal light period for reproduction lies above 12 hours (long-day plants) are more active photosynthetically or less active in removing or using the product under long-day illumination than under a 7-hour daily light exposure.

The growth data show that there was a rapidly increasing leaf area for photosynthesis and increased water loss in the clover, radish, and sunflower under long-day illumination. Notwithstanding the rapidity of growth and accompanying high rate of respiration for the necessary release of energy, the leaves of these plants showed the highest rate of increase in carbohydrates under equal periods of illumination. In addition, the extra hours of illumination resulted in an additional amount of food for increased growth. The demands for an ever increasing supply of water to replace that lost by transpiration, together with abundant materials for growth, resulted in every case in a root system the extent of which correlated well with the development of the tops. Under the short-day illumination less food was manufactured, growth of tops was retarded, and the root system was correspondingly abbreviated.

It seems that the dahlia and ragweed when grown under a long daily period of illumination, use the entire product of photosynthesis in promoting vegetative growth, the balance between the transpiring and absorbing system being well maintained. Under short-day illumination the dahlia accumulates a much greater relative amount of material in its fleshy roots. As pointed out by GARNER and ALLARD (**4**), this accumulation obviously indicates an excess of carbohydrates over current consumption. This surplus of carbohydrates is not due to increased photosynthetic activity but rather to the inability of the plant to utilize the carbohydrate, whether it be present in relatively large or small quantity. Since this portion of the food is not utilized by the growing plant, the result is a decreased extent of both root system and above-ground parts. The case of the ragweed is not quite so clear. It is a well established physiological fact, however, that respiration is high during the period of anthesis and much of the photo-

synthate furnished by the meager leaf area was undoubtedly used in supplying energy for this process. This interpretation is in agreement with that of GARNER and ALLARD (**4**) who conclude "that the duration of the daily illumination period not only influences the quantity of photosynthetic material formed but also may determine the use which the plant can make of this material."

NIGHTINGALE (**15a**) has shown that radish, salvia, buckwheat, and soy beans were limited in the synthesis of nitrates to other forms of nitrogen by a 7-hour day. Associated with relatively little assimilation of nitrates was an accumulation of carbohydrates within these plants when they were subjected to short-day illumination. Carbohydrates accumulated in the short-day plants, presumably because there was relatively little utilization of them in the synthesis of nitrates to other forms of nitrogen.

Summary

The relative development of roots and tops of eight species of plants grown under 7-hour and 15-hour daily illumination respectively, was determined. The plants were grown for about 7 weeks in soil in containers 30 x 30 x 80 cm. in size, which permitted of rather normal root development.

Red clover, radish, iris, and oats, all long-day plants as regards flowering, developed large tops and proportionately extensive root systems when subjected to the 15-hour day. Under short-day illumination the growth of both tops and roots was greatly retarded and approximately to the same degree. Their development was similar to that of the long-day plants when the latter were only 3.5 weeks old.

Sunflower, whose time of flowering is less modified by the length of day, developed in a manner similar to the preceding species.

Dahlia, the great ragweed, and cosmos are short-day plants. They attained their greatest size and greatest root development under the 15-hour day. Under short-day illumination the dwarfed tops were furnished with a correspondingly meager absorbing system, although more food was accumulated in the short-day dahlia. Thus in all cases development of the root systems was in direct correlation with the development of tops.

The effect of length of daily illumination upon photosynthetic activity showed that red clover, radish, and sunflower are either more active in photosynthesis or less active in removing or using the product under long-day illumination than they are under a 7-hour day. Great ragweed and dahlia were found to be more or less equally active photosynthetically under the two light periods, or less active in removal or use of the products under short-day illumination.

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LITERATURE CITED

1. Austin, C. L. Influence of pruning upon top and root development. California Agr. Exp. Sta. Rept. 1923: 210. 1923.
2. Chandler, W. H. Some results as to the response of fruit trees to pruning. Proc. Amer. Soc. Hort. Sci. **16**: 88–101. 1919.
3. Crist, J. W., and Stout, G. J. Relation between top and root size in herbaceous plants. Plant Physiol. **4**: 63–85. 1929.
4. Garner, W. W., and Allard, H. A. Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agr. Res. **23**: 871–920. 1923.
5. Gericke, W. F. Investigations of physiological processes related to root systems of plants. California Agr. Exp. Sta. Rept. 1922. 116–117. 1922.
6. Harris, F. S. The effect of soil moisture, plant food, and age on the ratio of tops to roots in plants. Jour. Amer. Soc. Agron. **6**: 65–75. 1914.
7. Jean, F. C., and Weaver, J. E. Root behavior and crop yield under irrigation. Carnegie Inst. Washington Pub. no. 357. 1924.
8. Johansson, Nils. Einige Versuche über die Einwirkung verschiedener Belichtung auf die vegetative Entwicklung von *Raphanus sativus* L. Flora **121**: 222–235. 1927.
9. Livingston, B. E. Note on the relation between growth of roots and of tops in wheat. Bot. Gaz. **41**: 139–143. 1906.
10. Long, F. L., Clements, F. E., and Haupt, C. R. Ecological measurement of photosynthate. Carnegie Inst. Washington, Year Book **24**: 322–323. 1925.
11. ————. Ecological measurement of photosynthate. Carnegie Inst. Washington, Year Book **25**: 352–354. 1926.
12. Lubimenko, V. N., and Szeglova, O. A. L'Adaptation photopériodique des plantes. Rev. Gen. Bot. **40**: 675–689. 1928.
13. Maximow, N. A., and Lebedincev, E. Über den Einfluss von Beleuchtunsgverhältnissen auf die Entwickelung des Wurzelsystems. Ber. deut. bot. Ges. **41**: 292–297. 1923.
14. ————, and Krasnosselsky-Maximow, T. Über den Einfluss von Beleuchtungsverhältnissen auf die Entwicklung und Tätigkeit des Wurzelsystems. Bull. Jard. Bot. République Russe **23**: 1–11. 1924.
15. Moeller, H. Beiträge zur Kenntniss der Verzwergung. Land. Jahrb. **13**: 167–173. 1884.

15a. Nightingale, G. T. The chemical composition of plants in relation to photo-periodic changes. Wisconsin Agr. Exp. Sta. Res. Bull. 74. 1927.

16. PFEIFFER, N. E. Microchemical and morphological studies of effect of light on plants. Bot. Gaz. **81**: 173–195. 1926.
17. REID, M. E. Growth of seedlings in light and in darkness in relation to available nitrogen and carbon. Bot. Gaz. **87**: 81–118. 1929.
18. TURNER, T. W. Studies of the mechanism of the physiological effects of certain mineral salts in altering the ratio of top growth to root growth in seed plants. Amer. Jour. Bot. **9**: 415–445. 1922. (See p. 442.)
19. WEAVER, J. E. Investigations on the root habits of plants. Amer. Jour. Bot. **12**: 502–509. 1925.
20. ————, KRAMER, J., and REED, M. Development of root and shoot of winter wheat under field environment. Ecology **5**: 26–50. 1924.
21. ZIMMERMAN, P. W., and HITCHCOCK, A. E. Root formation and flowering of dahlia cuttings when subjected to different day lengths. Bot. Gaz. **87**: 1–13. 1929.

SOME SEASONAL CHANGES IN THE TRACHEAL SAP OF PEAR AND APRICOT TREES

F. G. ANDERSSEN[1]

(WITH SEVEN FIGURES)

The sap in the tracheae of plants has long been known to contain various substances derived from the soil or from the surrounding tissues. Most of our knowledge of its composition has been obtained by study of the exudate (bleeding sap) from wounds during late winter (bleeding season) (CLARK, **4**; SCHROEDER, **23**; HORNBERGER, **11**; MOREAU and VINET, **16**). This has limited the study to a relatively short portion of the annual cycle and to comparatively few plants. Qualitative studies such as those of FISCHER (**9**, **10**) have shown that certain constituents, reducing sugars, occur in the tracheae at various times of the year. DIXON and ATKINS (**6**, **7**) carried out the most comprehensive study on tracheal sap yet reported. They obtained sap from tracheae by centrifuging wood from various parts of trees at different times of the year. They were able to follow the trend of changes in total electrolytes and sugars throughout the year and to estimate the concentrations of the sugars. MACDOUGAL (**13**) has also reported a few observations on the sugar content of the sap of pines.

The principal obstacle to the study of tracheal sap has been the difficulty in obtaining the large quantities needed for quantitative work with such a dilute solution. The sap has an electrolyte content approximating that of tap water, and organic substances are usually equally low. The only methods of obtaining the sap outside of the bleeding season have been to centrifuge portions of wood as done by DIXON and ATKINS (**6**) or to wash out the contents of the tracheae with water as done by MACDOUGAL (**13**) and MASON and MASKELL (**15**). Neither of these methods is suited to obtaining large quantities of sap, and both are liable to give sap which has been altered by contamination. An improved method has been described by BENNETT, ANDERSSEN, and MILAD (**2**) which reduces the liability of contamination and allows sap to be obtained from woody plants in quantities adequate for quantitative work.

It seems desirable to extend our knowledge of the composition and changes in the tracheal contents. The sap in the tracheae amounts to several per cent. of the weight of a woody stem. Our only accurate knowledge of it is limited to the bleeding season, during which period the tree is relatively inactive so far as the major activities of growth, absorption, trans-

[1] I take pleasure in expressing my grateful appreciation to Dr. J. P. BENNETT for his interest and valuable advice throughout this work.

piration, food manufacture, and translocation are concerned. Exact knowledge of the composition of, and changes occurring in, the tracheal sap, may aid materially in understanding these activities. The work reported here is a preliminary study of tracheal sap from pear and apricot trees. The sap was obtained by the gas displacement method (**2**) from the main branches of three-year-old Bartlett pear and Royal apricot trees growing in a heavy clay-loam soil. Each branch extracted consisted of two- and three-year-old wood. Extraction was always done within two hours after cutting the branches, it having been found that appreciable changes occurred in the sap if the branches were cut several hours before use. The branches were cut the same time of the day in order to avoid possible diurnal changes in the sap. Appreciable diurnal variation was noted by HORNBERGER (**11**) in the titratable acidity of sap from bleeding Birch trees. The sap obtained in the present work was colorless and clear or slightly cloudy, and of relatively uniform conductivity throughout the length of each branch.

Expressed sap, used for comparison with tracheal sap in part of the work, was obtained from portions of the same branches used for extraction of tracheal sap by grinding the wood after removal of the bark and pressing the ground tissue under a pressure of 400 kg. per sq. cm. The expressed sap thus obtained was clear but highly colored.

Buffer value and reaction of sap

Titration curves shown in figure 1 were prepared for tracheal and expressed sap of the Bartlett pear. Fresh 5-ml. portions extracted May 16 were titrated with 0.02 N H_2SO_4. The reaction of tracheal sap was determined colorimetrically, that of expressed sap electrometrically. From the curves it may be seen that the buffer value of expressed sap was about 25 times that of tracheal sap at the middle of May. The buffer value of both would doubtless vary during the year.

The low buffering of tracheal sap shows that extreme care must be exercised in determining its reaction. Contamination during extraction with very small amounts of vacuolar sap or other cell contents, or losses of dissolved gases might markedly affect it. Especial care was taken that all contamination from the cut surface of the wood through which the sap emerged was washed away before samples were retained for use. This was accomplished by determining the conductivity of succeeding ml.-portions of the sap as it was collected and discarding these until the conductivity became uniform. In the gas displacement method the sap is collected in a partially evacuated vessel, causing a loss of part of the dissolved gases. The gas of chief concern in relation to the reaction of the sap is carbon dioxide. This is known to be present in considerable amounts in the gases in the tracheae

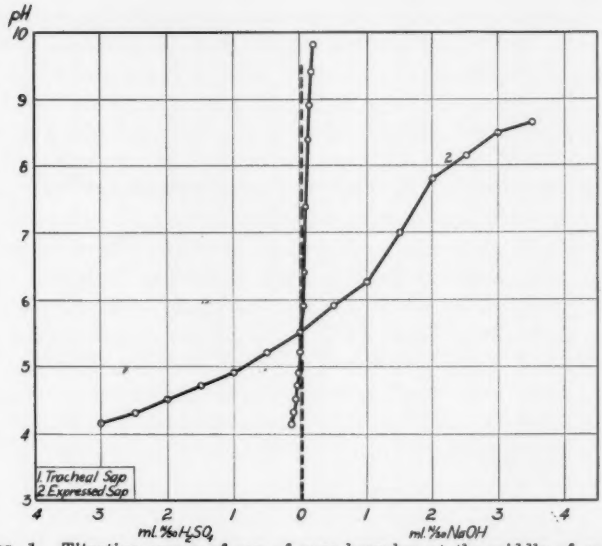

FIG. 1. Titration curves of sap of pear branches at the middle of spring.

(MACDOUGAL, **13**; CLARK, **4**) and tracheal sap would consequently be rich in it. It was not feasible in the present work to attempt to collect the sap with its full carbon dioxide content, and the losses during extraction would be variable. In order to make the samples comparable they were allowed to come into equilibrium with the air. The reaction of the sap as determined was then not necessarily the same as it was within the tracheae; but the variations in reaction found were due to substances other than carbon dioxide.

The expressed sap with its relatively high buffer value presented no especial difficulties in preparation.

The reaction of tracheal and of expressed sap from pear and apricot branches was determined at monthly intervals throughout a year. For each reported value sap was extracted separately from six branches, each from a different tree, the reactions determined separately and averaged. The results are shown in figures 2 and 3. The outstanding features of the curves for both apricot and pear is their convergence in the late winter and wide divergence later. For the pear, fig. 2, the period of approach lasts through spring, summer, and fall. During this period the curves are almost parallel. For the apricot the divergence of the curves began in midsummer and the period of close approach is relatively short. During one-half of the year in the apricot and one-third in the pear the tracheal sap was con-

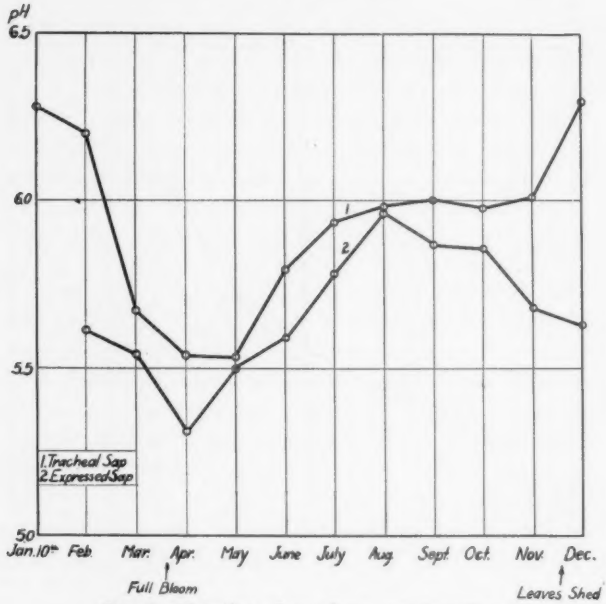

FIG. 2. Reaction of sap from pear branches.

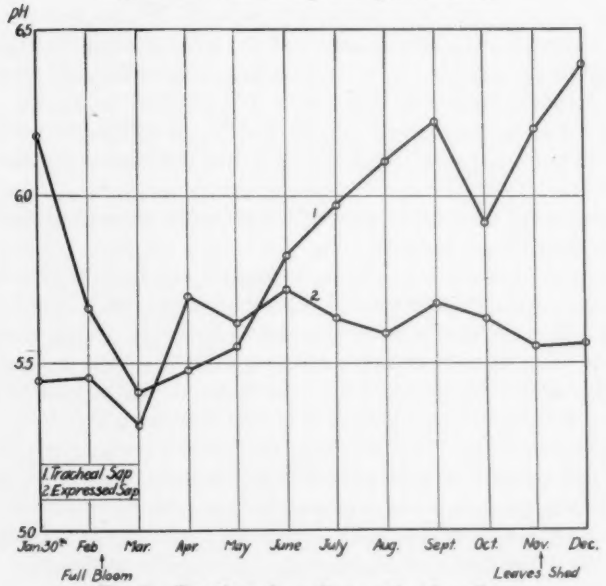

FIG. 3. Reaction of sap from apricot branches.

siderably more alkaline than the expressed sap. The maximum difference in reaction was in both cases about three-fourths of one pH. The total fluctuation in reaction was about 0.6 pH in the expressed sap of the pear and 0.7 pH in the tracheal sap, while in the apricot the expressed sap changed only about 0.4 pH and the tracheal sap about 1.0 pH. The winter divergence in the reaction was then due about equally to changes in expressed and tracheal sap in the pear, while in the apricot it was due more largely to changes in the tracheal sap. The trends of the curves suggest that during the active growing season the reaction of the tracheal sap is strongly influenced by that of the surrounding tissues, while during the dormant season this influence is absent. It seems probable that the increased acidity in early spring is due to an increase in organic acids in the sap. During the period of renewed growth respiration is very active and may result in a general increase in the concentrations of organic acids present in the tissues. HORNBERGER (**11**) found that the titratable acid in "bleeding" sap increased during the blossoming period and later decreased.

Inorganic constituents

It has long been known that the tracheal sap obtained from bleeding plants contained salts. SACHS (**22**) detected the presence of K, Ca, PO_4, and SO_4. HORNBERGER (**11**) studied quantitatively the exuded sap of the Birch and Hornbeam and determined the amounts of Na, Ca, and Mg present. PFEFFER (**18**) pointed out that the composition of the sap is not constant. The study of bleeding sap, of course, gives a very incomplete picture, leaving the composition and changes in the sap during most of the year entirely unknown. The most comprehensive investigation of tracheal sap is that of DIXON and ATKINS (**6, 7**) mentioned previously. The contamination of the sap by materials from the injured cells at the cut surface of the branch during the extraction of tracheal sap by the centrifuge method makes it seem probable that the results of DIXON and ATKINS are somewhat too high.

TOTAL ELECTROLYTES

The specific resistance of the sap was determined at monthly intervals throughout the year. The sap used was of the same lots as used in the determinations of reaction. Each value reported was the average of six determinations, each on sap from a single branch. The results are presented in figures 4 and 5. Both kinds of trees showed a minimum concentration of electrolytes during the dormant season, the pears at about midwinter and the apricots at the time of leaf fall. From very early spring there was a very rapid increase in concentration, reaching a maximum at or shortly after full bloom; after this maximum there was again a gradual decrease in

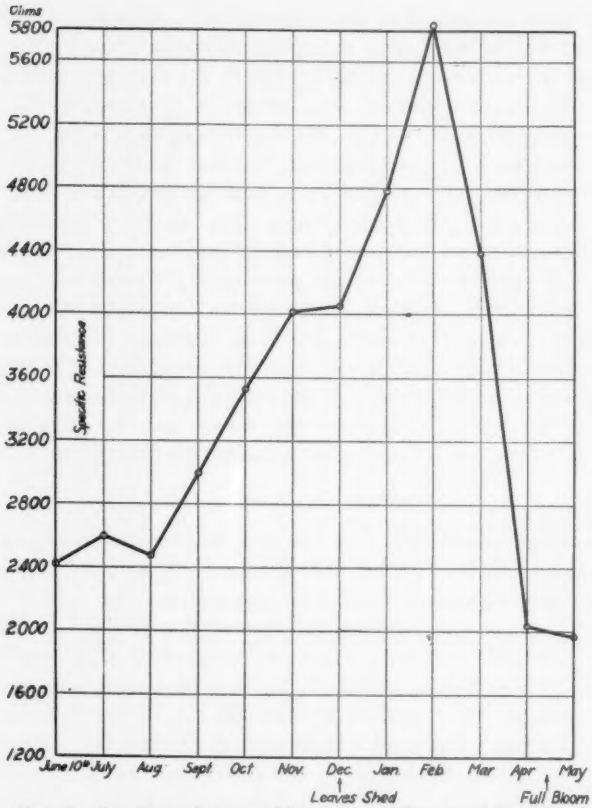

FIG. 4. Specific resistance of tracheal sap from pear branches.

concentration through the summer. The results in general agree with those of DIXON and ATKINS (**6, 7**) and of SCHROEDER (**23**).

The rapid increase in concentration in early spring may have been due to the action of one or more of several factors: the increase occurred at a time when the differentiation of tracheae was beginning, and the contents of such cells became part of the transpiration stream; at the same time the rate of respiration was increasing and the resultant hydrolysis and oxidation of cell constituents may have increased the concentration of diffusible ions in the parenchyma surrounding the tracheae, thus favoring the diffusion of more ions into the vessels. It is also possible that the permeability of the protoplasts may have increased during this period of increased activity, allowing an influx of solutes from the concentrated sap of the parenchyma cells into the tracheae. The roots were also actively growing at this

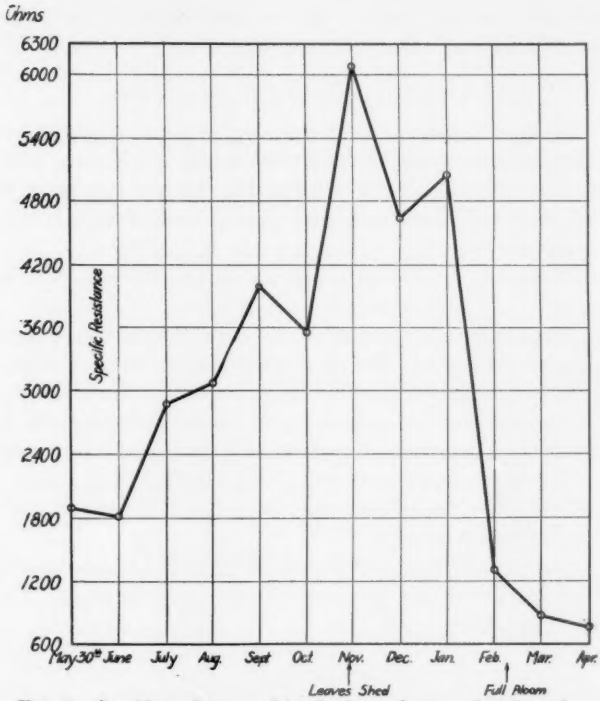

FIG. 5. Specific resistance of tracheal sap from apricot branches.

period, and a rapid absorption of ions from the soil solution would presumably be reflected in the concentration of the tracheal sap.

At or shortly after the blossoming season the leaves began to develop, and transpiration became more and more rapid. This would apparently cause a relatively greater intake of water than of salts, and result in a large dilution of the electrolytes in the transpiration stream. During the summer a decrease of root activity would further reduce the salt intake while the transpiration remained large. The decrease in concentration of electrolytes may be found to continue into early winter, if the tracheae have not yet reached their maximum water content by the time all the leaves have been shed.

The irregularities in the curves seem to be related, to a certain extent, to moisture conditions of the atmosphere and the soil. During rainy weather or just after an irrigation the concentration of the tracheal sap was generally less than during a dry period. Temperature changes also may exert some effect in this respect; MOREAU and VINET (**16**), working with

bleeding sap of the vine, found a fall in temperature to be followed by a decided increase in concentration of the sap.

Individual inorganic constituents

These were determined in the tracheal sap of the pear only at two times in the year at approximately the points of lowest and highest total concentration of electrolytes as indicated by the curves. Large amounts of tracheal sap were collected from pear branches on November 10, and on May 10 following.

The methods used in analyzing the sap are briefly indicated in the following:

Ca, Mg, and K were determined on the same sample, using 150–200 ml., depending upon the season. The Ca was determined as calcium oxalate, the Mg as $MgNH_4PO_4$ and the K as K_2PtCl_6.

The sulphate was determined as $BaSO_4$ in 100–150-ml., and the Cl as AgCl in 150–200-ml. samples. Phosphate was determined by a modification of the colorimetric method of Atkins (**1**) in 0.2–25.0-ml. samples. For Fe a slight modification of the colorimetric method of Marriott and Wolf (**14**) was used on 50–100-ml. samples.

An analysis of a 1:1 water extract of the soil on November 10 at 18 inches and at 36 inches deep was made by the same methods. The results are shown in table I.

TABLE I

Inorganic constituents in tracheal sap of pear and in the soil extract

	Depth	Soil extract November 10	Tracheal sap November 10	Tracheal sap May 10
	inches	*ppm.*	*ppm.*	*ppm.*
Ca	18	18.0	16.6	84.7
	36	7.5		
Mg	18	7.2	0.8	23.5
	36	5.4		
K	18	140.9	23.6	59.6
	36	230.0		
Fe	18	1.8	1.0	2.1
	36	1.0		
SO_4	18	37.2	8.3	31.8
	36	30.0		
Cl	18	14.0	3.2	4.5
	36	9.5		
PO_4	18	1.0	10.6	25.2
	36	trace		

Without exception there was a very marked increase in concentration between November and May of the substances determined. Several of the ions on May 10, Ca, Mg, and PO_4, even far exceeded the concentration in the soil extract. Unfortunately a soil extract on May 10 was not obtained. Probably the concentration of the soil extract in the spring was higher than in November, but whether the difference would be closely related to the difference in sap concentration remains to be determined.

In the case of phosphate there appears to be an immediate source other than the soil for the relatively large amount found in the sap in the spring. The results of phosphate determinations at monthly intervals are shown in table II. The phosphate content in pear sap increased to fifteen fold between February 10 and May 10. This relatively large increase in concentration of an ion relatively low in concentration in the soil suggested the possibility that much of the phosphate present in the sap may have come from the surrounding tissues. This suggestion was more strongly supported by examination of apricot sap in which the increase from January 3 to March 30 was 171 times.

TABLE II

PHOSPHATE CONTENT OF TRACHEAL SAP

DATE	PEAR	DATE	APRICOT
	ppm.		*ppm.*
Aug. 10	12.7		
Sept. "	11.6		
Oct. "	6.9		
Nov. "	10.4		
Dec. "	2.3		
Jan. "	1.8	Jan. 3	0.9
Feb. "	1.8	Jan. 31	10.7
Mar. "	1.7	Mar. 1	74.0
Apr. "	22.5	Mar. 30	154.0
May "	25.2	Apr. 30	37.5

It is definitely known that phosphorus is utilized during the synthesis of starch, proteins and perhaps other complex substances, and that on hydrolysis of such substances the phosphorus may again be set free as phosphate. NORTHROP and NELSON (**17**), making a quantitative study on phosphorus content of starch, showed the presence of 0.6 per cent. of phosphorus, 95–97 per cent. of which is in organic combination.

Further evidence favorable to the view that the above increase in phosphate may be due, at least in part, to release from an organic source was found by CAMERON (**3**) in some carbohydrate studies on citrus trees; the

evidence seemed conclusive that as the amount of starch in the wood increased through the season the amount of PO_4 extractable with water decreased. It seems probable, then, that part of the phosphate content of the tracheal sap may have come from the surrounding cells in which stored starch was being hydrolyzed in the late winter.

Organic constituents

Free reducing substances and sugars

Determinations of the reducing power, before and after inversion, of the sap from the pear were made on the same lots of sap used in following the total electrolytes. Each point on the curves represents the average of six determinations. The picric acid colorimetric method essentially as described by Willaman and Davison (**25**) was used in determining reducing power. The method was found to give very consistent results, and was well adapted to this work because only 2 ml. of sap was required for each determination. The results are shown in figure 6. The designation "free reducing substances" is used instead of sugars, because it appears highly probable that not all the reducing substances present were sugars; but sucrose appears to be an accurate designation for the inversion product, because the same values were obtained when hydrolyzing with picric acid according to the cited method (**25**), as with invertase.

The most striking fact shown by the curves is the very marked increase in both reducing substances and sucrose shown during very early spring. Then the sucrose rapidly fell to practically zero shortly after full bloom. The reducing substances continued to increase at first, seemingly at the expense of the sucrose, up to a maximum at full bloom, and fell exceedingly rapidly to zero within the next month. There was a period of two to three months in summer during which the sugars were present, if at all, in indeterminable amounts. In early fall there again appeared a moderate amount of reducing substances and a small amount of sucrose. From this time on both the curves slowly rose through winter until they again reached the vernal maximum.

Dixon and Atkins (**6, 7**) also report finding a great increase in sugar content in early spring. In the deciduous and in the evergreen trees used by them, the sucrose more often exceeded the reducing substances. Their reducing substances at the vernal maximum consisted of hexoses and maltose; later in the season the maltose was absent. Fischer (**9, 10**), using microchemical methods, found a similar accumulation of reducing substances in the tracheae at the time of blossoming. Schroeder (**23**) found in the exudate from a tapped Birch tree within a period of seven weeks from early to late spring, a great decrease in concentration of sugars.

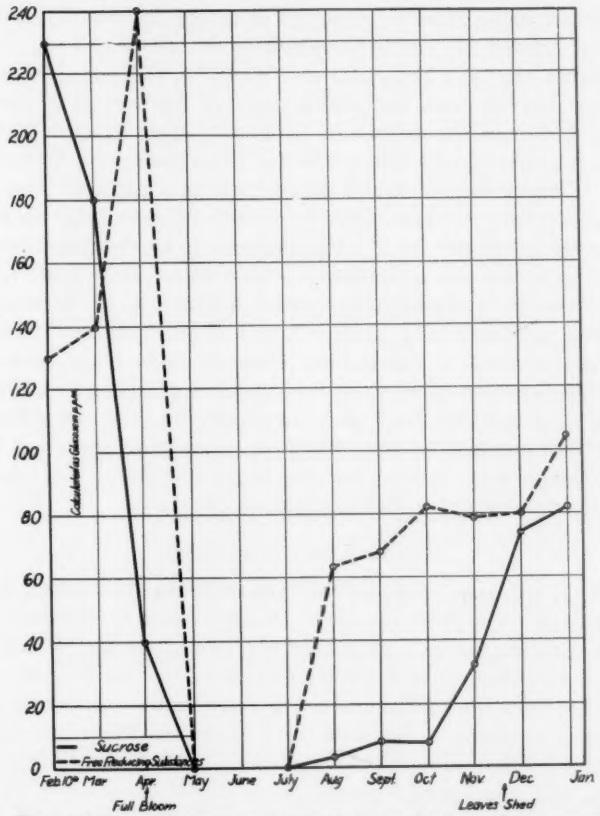

FIG. 6. Free reducing substances and sucrose in tracheal sap from pear branches.

MACDOUGAL (**13**) reports the presence of appreciable amounts of reducing substances in sap extracted from the wood of Monterey Pine. The method of obtaining sap was essentially that of water displacement described by BENNETT, ANDERSSEN, and MILAD (**2**). An aqueous solution of dye was forced through the stem under a pressure of less than one atmosphere. The extractions reported were from June to October and show quite wide variations in sugar content. Since no mention is made of precautions taken to exclude such sources of error as mixture with the displacing water which may forge ahead of the dye (EWART, **8**; PRIESTLEY and ARMSTEAD, **20**), or with substances from injured cells around the point of extraction (BENNETT, ANDERSSEN, and MILAD, **2**), it is not clear that the variation in sugar content reported is not in part a result of the conditions of extraction. A

similar result would arise in extraction of sap by the centrifuge method of DIXON and ATKINS (**6**) and in the obtaining of "bleeding" sap.

PRIESTLEY (**19**) and PRIESTLEY and WORMALL (**21**) considered it wrong to suppose that the great increase in sugar in the trachea in early spring arose directly from the hydrolysis of starch; they believed that it came from the contents of cells differentiating into vessels. However, the great increase in sugar content found in the present work took place so early in the spring that one can scarcely consider differentiation to have been rapid enough to account for it. Furthermore, in late spring differentiation of cells into vessels was undoubtedly taking place much more rapidly, so that, even though the transpiring surface and the rate of the transpiration stream had simultaneously increased, one might have expected at least determinable amounts of sugars from this source to be found in the tracheal sap. It has also been determined that the time when the sugars drop to a minimum is likewise the time when the starch has been completely hydrolyzed and has disappeared from the more active outer section of the wood (**10**). It seems that, in the pear, the sugar content of the tracheal sap bears a close relation to the disappearance of starch.

NITROGENOUS CONSTITUENTS

The total nitrogen present in the tracheal sap was found to consist almost entirely of organic nitrogen. Nitrate could be detected with the diphenylamine reagent only after concentration of the sap. On November 10 the sap contained 60 ppm. of total nitrogen, and on May 10 163 ppm. of which 110 ppm. were present as amino nitrogen by VAN SLYKE'S method and 49 ppm. as amide by the method of SACHSSE. There was apparently very little or no protein present. SCHROEDER (**23**), however, found as high as 33 ppm. of heat-coagulable protein in the sap of the Birch in April.

TOTAL SOLIDS

The usual Beckmann apparatus was found unsatisfactory for determining total concentration of solutes on account of the dilution of the sap. Determinations of refractive index at 25° C. were made with an Abbé's refractometer on the same sap as was used for electrical conductivity measurements. The data are represented in figure 7. It could not be expected to correlate these curves for total solids with those for total electrolytes, reducing substances and sucrose, because there are apparently other substances present for which analyses were not made. Thus, it has been found in the sap bleeding from a vine that the amount of organic acids present exceeds the total amount of all other organic substances (**21**). The higher values of refractive indices during the winter months coincide roughly with the increased amounts of organic and inorganic constituents

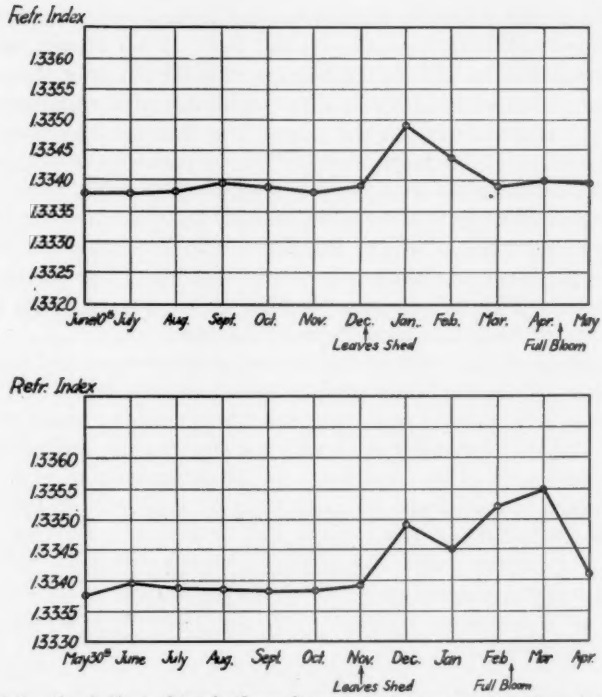

FIG. 7. Refractive indices of tracheal sap from pear (upper curve) and apricot branches.

found in the sap during that period. But the general lack of parallelism between the curves for refractive indices and those for total electrolytes, reducing substances and sucrose indicate that other substances than these were affecting the results.

It is not possible to compare these results directly with those obtained for other deciduous trees, *Acer macrophyllum* and *Ulmus campestris*, by DIXON and ATKINS (**6, 7**), since they determined the freezing point depression of the sap which is dependent upon the total solutes. They report a low osmotic concentration during late autumn and winter, whereas the pear and apricot both showed an increase in total solids in winter. This may be a difference shown by the different plants used, or it may quite possibly be due to the methods employed.

Radial distribution of sap constituents

STRASBURGER (**24**) and later DIXON (**5**) pointed out that the interpretation so often given to ringing experiments rests on the assumption that it

is possible to ring a branch without causing injury to the tracheae of the ring of wood adjoining the cambium and bark. Even though the ring of bark may be removed without directly injuring the tracheae, the wounding and exposure to the air is sufficient to cause injury, resulting in the clogging of such tracheae with air and gums. Furthermore, the outer tracheae are considered to be the most important in the transport of solutes up the tree, so that interference with the flow through these would be sufficient to retard or prohibit growth of buds above the ring, or to cause the starvation of the tree as a whole when the trunk was ringed. Because of the divergence of opinion on this matter, it was considered of interest to actually determine the difference in concentration of the sap collected from the outer tracheae and of that from the inner tracheae.

The method of the extraction of the sap is the same as that referred to earlier (**2**). When the branch was prepared as described there, a sharp-edged metal tube, about 1 inch long, was driven into the peeled end of the branch. The tube selected was of such a diameter as to include all of the cross-sectional area of the branch except the outer annular ring. During the extraction, the sap from the inner rings of wood was led by the metal tube into one collecting vessel while that from the outer ring dropped directly into another vessel. As in the previous extractions, the first few cubic centimeters collected in the tubes were discarded on account of contamination. A few of the results obtained by this method on pear branches are shown in table III.

TABLE III

RADIAL DISTRIBUTION OF SAP CONSTITUENTS IN THE PEAR

DATE	pH		FREE REDUCING SUBSTANCES		TOTAL REDUCING SUBSTANCES AFTER HYDROLYSIS		SPECIFIC RESISTANCE	
	OUTER	INNER	OUTER	INNER	OUTER	INNER	OUTER	INNER
			ppm.		*ppm.*		*Ohms.*	*Ohms.*
Feb. 24, '27	6.3	6.6	180	none	350	trace	4320	6550
	6.2	6.5	160	none	450	trace	3590	9520
April 4, '27	5.9	6.2	.....	.....	409	trace	2370	5000
	5.8	6.1	.....	.....	500	trace	2240	5030
May 9, '27	5.3	5.5	132	none	409	none	1615	2420
	5.3	5.5	200	none	240	none	1640	2380

Branches 3 years old were used for the results reported in the table in order to obtain large amounts of sap. However, similar results were obtained when one-year-old shoots of the previous season's growth were used. In this latter case the shoots had a diameter of 1 to 1.5 centimeters, and the

area enclosed by the metal tube comprised about half of the cross-sectional area of the shoot.

Nitrogen determinations were also made on outer and inner tracheal sap from 3-year-old branches on April 4. The outer sap contained 65.5 ppm. while the inner sap contained 26 ppm. of total nitrogen.

The specific resistances in table III show very definitely that the sap in the outer tracheae is much more concentrated in electrolytes than that in the inner tracheae. The concentration of the sap obtained from the inner or outer tracheae by the above method will undoubtedly depend upon the fraction of the whole cross-section which is being considered as inner or outer tracheae. This may possibly account for the differences found among different branches taken on the same date.

MACDOUGAL (**13**) reports differences in the sugar content of sap extracted from the different annual rings of the Monterey Pine. The results reported in this paper are in general agreement with those of MACDOUGAL; a more concentrated sap was obtained from outer than from inner annual rings. MACDOUGAL'S extractions were made from June to October while the pear saps were extracted from February to May. While too few data are available for comparison of these results, it may be briefly pointed out that the sugar concentrations found by MACDOUGAL in pine sap were usually very much higher than those found in pear sap, and that a much wider variation of concentrations was reported for the former. It does not seem probable that pine sap in the summer season would be so much more concentrated in sugar and free reducing substances than pear sap in the spring. DIXON and ATKINS (**7**) did not find any greater concentrations of sugar in the tracheal sap of evergreens than of deciduous trees. It is suggested that much of the reducing substances found by MACDOUGAL came from the injured living cells adjoining the bored hole through which the sap was extracted. As pointed out by BENNETT, ANDERSSEN, and MILAD (**2**) this injured surface has a very marked effect on its sugar content.

With regard to the sugar and free reducing substances it may be seen that the outer tracheae contained practically all of these throughout the period of observation. When all the sugar was found in the outer tracheae during early spring, it was thought possible that the situation might become reversed in late spring, when all the starch in the outer ring would have been hydrolyzed and the starch from the inner rings would be rapidly hydrolyzing. This was not found to be the case; on May 9 sugar was found only in the outer tracheae after starch had completely disappeared from the outer ring of wood. This fact seems to show that the starch in the inner rings, when hydrolyzed, does not pass into the adjacent tracheae but is

transferred along the medullary rays towards the outer tracheae into which it is delivered.

The sap from the outer tracheae is also invariably more acid than that from the inner. This seems probably related to the fact that the outer tissues are the younger and more active metabolically and are close to the very active cambium and layers of differentiating cells.

Summary

1. The preliminary data reported in this paper include determinations of buffer value, reaction, content of electrolytes, individual inorganic constituents, free reducing substances, sucrose, nitrogenous compounds, and total solids in tracheal sap of pear or apricot branches at various times during the year. The radial distribution in tracheal sap of acidity, total electrolytes, and free reducing substances before and after inversion is also shown for pear branches during the early spring.

2. The buffer value of tracheal sap at the middle of spring was found to be about one twenty-fifth that of expressed sap from the same tissues.

3. Tracheal and expressed sap reached maximum acidity in early spring, then fell gradually to minimum acidity in winter. The change from minimum to maximum acidity in early spring was very rapid and coincided with the resumption of growth.

4. Total electrolyte concentration and increased acidity of the tracheal sap ran parallel. The lowest concentration occurred in winter, followed by a very rapid increase in early spring, at the time of growth resumption. A gradual decrease took place during the summer and fall.

5. Individual inorganic constituents in the tracheal sap showed a large increase in concentration from early winter to early spring.

6. Phosphate in the tracheal sap showed a very large increase between late winter and early spring. It is suggested that this increase is due in large part to phosphate set free in the hydrolysis of starch.

7. Free reducing substances and sucrose showed a high concentration in late winter and early spring, a rapid decrease in late spring, an indeterminable low amount during summer, and a gradual rise during fall and winter.

8. The total solids, determined refractometrically, were found to rise during the winter months and decline during the spring, and then remain at a relatively uniform level during the remainder of the year.

9. The free reducing substances and sucrose were found to be limited to the outer annual ring in three-year-old pear branches during late winter and spring. Total electrolytes were about twice as concentrated in the outer

annual ring as in the inner rings. The sap from the outer annual ring was more acid than that from the inner rings.

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LITERATURE CITED

1. ATKINS, W. R. G. The rapid determination of available phosphate in soil by the coeruleo-molybdate reaction of Denigès. Jour. Agr. Sci. **14**: 192–197. 1924.
2. BENNETT, J. P., ANDERSSEN, F. G., and MILAD, Y. Methods of obtain-in tracheal sap from woody plants. New Phytol. **26**: 316–323. 1927.
3. CAMERON, S. H. Unpublished data.
4. CLARK, W. S. Observations upon the phenomena of plant life. Massachusetts State Board of Agr. Ann. Rept. **22**: 204–314. 1875.
5. DIXON, H. H. The transpiration stream. Pp. 52–53. Univ. of London Press. 1924.
6. ————, and ATKINS, W. R. G. On the constituents and concentration of the sap in the conducting tracts, and on the circulation of carbohydrates in plants. Sci. Proc. Roy. Dub. Soc. n.s. **14**: 374–392. 1915.
7. ————. On the composition of the sap in the conducting tracts of trees at different levels and at different seasons of the year. Sci. Proc. Roy. Dub. Soc. n.s. **15**: 51. 1916.
8. EWART, A. J. On the ascent of water in trees. Phil. Trans. Roy. Soc. London B **198**: 41–85. [1904] 1906; and B **199**: 341–392. [1907] 1908.
9. FISCHER, A. Glycose als Reservestoff der Laubhölzer. Bot. Zeitg. **46**: 405–417. 1888.
10. ————. Beiträge zur Physiologie der Holzgewächse. Jahrb. wiss. Bot. **22**: 73–160. 1890.
11. HORNBERGER, R. Beobachtungen über den Frühjahrssaft der Birke und Hainbuche. Forstliche Blätter. **1887**: 321; abstr. in Bot. Centralbl. **33**: 227–229. 1888.
12. MACDOUGAL, D. T. Reversible variations in volume, pressure, and movements of sap in trees. Carnegie Inst. Washington, Pub. no. 365. 1925.
13. ————. The hydrostatic system of trees. Carnegie Inst. Washington, Pub. no. 373. 1926.
14. MARRIOTT, W., and WOLF, C. G. L. The determination of small quantities of iron. Jour. Biol. Chem. **1**: 451–461. 1906.

15. Mason, T. G., and Maskell, E. J. A study of diurnal variation in the carbohydrates of leaf, bark, and wood, and of the effects of ringing. Ann. Bot. **42**: 189–253. 1928.
16. Moreau, L., and Vinet, E. Sur la composition des pleurs de vigne. C. R. Acad. Agr. France **9**: 554–557. 1923.
17. Northrop, J. H., and Nelson, J. M. The phosphoric acid in starch. Jour. Amer. Chem. Soc. **38**: 472–479. 1916.
18. Pfeffer, W. Physiology of plants. Translation by A. J. Ewart. Vol. I: 262. University of Oxford Press. 1900.
19. Priestley, J. H. The first sugar of photosynthesis and the rôle of cane sugar in the plant. New Phytol. **23**: 255–265. 1924.
20. ————, and Armstead, Dorothy. Physiological studies in plant anatomy. II. The physiological relation of the surrounding tissue to the xylem and its contents. New Phytol. **21**: 62–80. 1922.
21. ————, and Wormall, A. On the solutes exuded by root pressure from vines. New Phytol. **24**: 24–38. 1925.
22. Sachs, J. On the physiology of plants. Translation by H. M. Ward. Page 274. University of Oxford Press. 1887.
23. Schroeder, J. Die Frühjahrsperiode der Birke (*Betula alba* L.) und des Ahorn (*Acer platanoides* L.). Landw. Versuchsst. **14**: 118–146. 1871.
24. Strasburger, E. Ueber den Bau und die Verrichtungen der Leitungsbahnen in den Pflanzen. Histologische Beiträge **3**: 591–595, 877–928. 1891.
25. Willaman, J. J., and Davison, F. R. Some modifications of the picric acid method for sugars. Jour. Agr. Res. **28**: 479–488. 1924.

PHYSICAL MEASUREMENTS OF THE WINTER WHEAT PLANT AT VARIOUS STAGES IN ITS DEVELOPMENT

GEORGE JANSSEN

(WITH FOUR FIGURES)

Introduction

Winter hardiness of the wheat plant has in the main been associated with the hereditary characters of the plant. This is evidenced by the results of comparative winter wheat variety tests conducted at various experiment stations. It has been noted that modifications of this winter hardiness factor can be brought about by subjecting the same plant to different environmental conditions. This is evidenced by the work of CHANDLER (**1**), ROSA (**18**), SALMON (**20**), and NEWTON (**14**). In view of these results it might be expected that plants of the same variety of wheat, but having attained different stages of seedling development would respond differently to freezing temperatures. With this in mind a study was made of: (1) the effect of rate of thawing of frozen wheat plants at various stages of seedling growth; (2) the variations in sap concentration of winter wheat plants that had reached various degrees of development, as a result of periodical fall seedings and, (3) the relationship between the sap concentration as measured by freezing point depression, actual chemical composition, and winter hardiness.

Method

This study was conducted on a pure line of Turkey wheat, Wisconsin Pedigreed no 2. The wheat plants used for chemical purposes were taken from 1/40 acre test plats supervised by Professor B. D. LEITH, on the University farm. The seedings were made on or near the following dates: August 15, September 1, September 15, October 1, and October 15. The percentages of winter killing were determined for these various dates of seeding by making actual plant counts. These percentages of winter killing for 1923–1925 are presented in fig. 1. Material for miscellaneous freezing experiments was started in earthenware pots at the same time the field seedings were made. These potted plants were stored at the end of the fall growing season in a cool part of the greenhouse until used in the winter.

CHEMICAL ANALYSIS

All methods of chemical analyses were essentially the same as given in another paper (**7**).

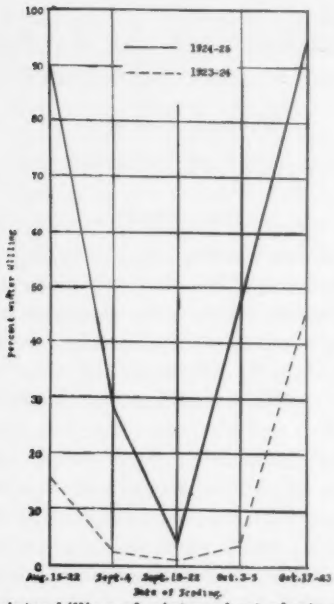

FIG. 1. Percentage of winter killing of winter wheat plants which occurred during the winter of 1923–24, and 1924–25, on five periodic seedings made during the fall season.

PHYSICAL METHODS

Samples of plants from which sap was expressed and used for physical and chemical analysis were collected and packed in jars surrounded with ice. This was done in the field when the collections were made. The samples were always kept cooled to or near 0° C. The extraction equipment was cooled in an ice bath previous to the extraction of the sap. The technique of expressing the plant sap and the determination of the freezing point was that described by GORTNER (**4**). As soon as collections were taken to the laboratory, the adhering soil was removed from the roots and the dead leaves cut away. The samples were then ground with an ordinary meat grinder equipped with a fine bur (peanut bur) which ground the tissue into a very fine pulp. Duplicate ten-gram samples were then taken for moisture determination. The tissue was further ground in a mortar. After grinding, 100 grams of the pulp were placed in a suitable cylinder with a two-inch bore, which was provided with a piston to which pressure could be applied by means of a hydraulic press. A pressure of 35,000 pounds was applied to the entire cylinder of tissue, which was sufficient to extract the sap, leaving the pulp in a very dry condition.

The sap was then centrifuged for five minutes at 22,000 revolutions per minute. The depression of the freezing point, total solids, and specific gravity were then determined on the centrifuged sap.

Rates of thawing

Much work has been done on the rate of thawing (**12, 13, 19**) of frozen plant tissues in an effort to determine whether or not this is a factor in winter hardiness. West (**22**) believes that at the freezing point of the plant tissue, water passes out of the cells and ice forms in the intercellular spaces. In tender fruits and vegetables when thawing occurs slowly this water will pass back into the cells if the cell walls are not ruptured. If thawing is rapid the water is not taken into the cells and the tissue dies, due to lack of water. Müller-Thurgau (**13**) obtained injury in the pear and apple only when he employed rapid thawing. Chandler (**1**) obtained similar results with ripe apples and pears. He found also that slow thawing of lettuce was more beneficial than rapid thawing. Wiegand (**23**) noted no injurious results from the rapid thawing of the frozen buds and twigs from several species of shrubs and trees. Gardner, Bradford, and Hooker (**2**) conclude that rapid thawing has no serious effect on frozen plant tissue.

In 1923 and 1924 the writer studied the effect of differential rates of thawing frozen wheat plants. Hardened winter wheat plants were taken from the field when the soil was frozen. At this time a recording thermograph registered the soil temperature at $-5°$ C. in 1923 and $-20°$ C. in 1924 at a depth of two inches below the surface. Blocks of soil two feet square and ten inches deep containing about 30 plants, were removed from the field to the greenhouse. Four samples each were taken from the plots sown on the following dates, nantely, August 15, September 4, September 22, and October 5. The plants in the blocks of earth were thawed out at the following temperatures, 33°, 27°, 15°, and 5° C. Recovery data are presented in table I.

As shown in table I, of the plants frozen at $-5°$ C., only those from the third and fourth seedings recovered in excellent condition at all four thawing temperatures. Plants from all the seedings responded equally well when thawed at 5° C. In practically all cases plants from the second, third and fourth seedings, which had been frozen at $-20°$ C. recovered and lived when they were thawed at a temperature of 5° C., as opposed to a total loss from all seedings which occurred with higher thawing temperatures. The plants in the field from which the above samples were taken survived the winter with a minimum of killing, which seems to indicate that under outdoor conditions the gradual daily increase in temperature, noted by the daily temperature chart, allowed these plants to become adjusted to the environ-

TABLE I

GENERAL EFFECT ON THE WHEAT PLANT OF RAPID AND SLOW THAWING PLANTS TAKEN IN FROZEN BLOCKS OF EARTH FROM THE FIELD EXPERIMENTAL PLATS, AND THEN SUBJECTED WHILE THAWING TO THE TEMPERATURES NOTED

TEMPERATURE OF THAWING	FIRST SERIES		SECOND SERIES	
	SEEDING DATE	1923 FROZEN AT −5° C. RECOVERY	SEEDING DATE	1924 FROZEN AT −20° C. RECOVERY
33° C.	Aug. 15	Very poor	Aug. 15	Killed
	Sept. 4	Fair	Sept. 4	Killed
	Sept. 22	Excellent	Sept. 22	Killed
	Oct. 5	Excellent	Oct. 5	Killed
27° C.	Aug. 15	Poor	Aug. 15	Killed
	Sept. 4	Excellent	Sept. 4	Killed
	Sept. 22	Excellent	Sept. 22	Killed
	Oct. 5	Fair	Oct. 5	Killed
15° C.	Aug. 15	Good	Aug. 15	Killed
	Sept. 4	Excellent	Sept. 4	Very poor
	Sept. 22	Excellent	Sept. 22	Very poor
	Oct. 5	Excellent	Oct. 5	3 plants lived
5° C.	Aug. 15	Excellent	Aug. 15	Killed
	Sept. 4	Excellent	Sept. 4	Fair
	Sept. 22	Excellent	Sept. 22	Fair
	Oct. 5	Excellent	Oct. 5	Killed

ment. This adjustment apparently was not possible for plants thawed in the greenhouse.

It is interesting, in connection with these experiments on rate of thawing, to note the effect on the plants of a second severe freeze. After the plants from the first experiment had recovered and had reached a growth condition similar to that existing before the first freezing, they were taken from their respective temperature rooms and placed under an air temperature of about −10° C. The plants taken from the room having a temperature of 5° C. were not killed by this second freezing, whereas those from higher temperatures were killed. This experiment was repeated, using plants from sowings made on different dates in 10-inch earthenware jars. These jar cultures were then stored late in the fall in a cool part of the greenhouse. The plants remained in excellent condition and were used for various experiments at intervals during the winter. In January, eight pots from each of the series sown on four dates were placed out of doors where the air temperature approximated −20° F. Two representative pots from each date series were taken into the greenhouse after 4-hour exposure and a

similar two after eight hours. These were thawed at 22° C. The remaining four pots of each date series were left outdoors until spring. The experiment was repeated one week later.

Plants which were taken into the greenhouse and thawed at a temperature of 22° C. were killed, whereas those which remained out of doors till spring recovered (see fig. 2). Immediately after the plants thawed at 22°

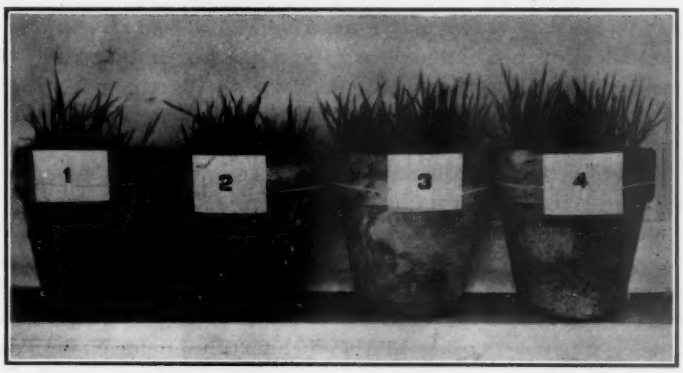

FIG. 2. Recovery of winter wheat plants from four dates of seeding (Aug. 15, Sept. 4, Sept. 22, and Oct. 5) after having been subjected to an outdoor temperature of – 20° F. in January, and allowed to remain outdoors until spring. These and other similar plantings were stored in a cool part of the greenhouse where the plants remained dormant. In January, when the thermometer registered – 20 F., these and other similar series of plants were placed outdoors. At the end of four and eight hour periods several series were taken into the greenhouse and allowed to thaw at a temperature of 22° C. Those plants taken into the greenhouse were all killed, while the series which remained outdoors until spring recovered in excellent condition.

C. were placed in the warm room, the leaves became flaccid and wilted. Moisture appeared on the epidermis and later evaporated, leaving the plant in a desiccated condition. The lack of available moisture in the frozen soil further tended to reduce the plant to a state of dryness from which it was not able to recover. The plants which remained out of doors were later covered with a blanket of snow, and remained in this condition until spring. The slow daily increase in outdoor temperature caused these plants to be thawed slowly. Under these conditions the plants came through the winter in excellent condition. It will be seen from fig. 2 that there is little difference in the recovery of the plants from the various dates of seeding.

A specific explanation for the recovery of the plants which remained outdoors until spring and the reason for the death of the plants which were taken into the greenhouse cannot be given. Work on protein precipitation as caused by freezing (**3, 7, 21**) may be suggestive. Since purified proteins

free from salts and acids are not precipitated by frost (10), we would not expect precipitation of proteins to occur in plants unless water was first removed from the tissue and the concentration of salts and acidity thereby increased. It seems that the death of these plants is caused by the loss of water, but whether the final result is directly or indirectly attributable to this condition cannot be stated. The importance of the rôle which the carbohydrate compounds play in protecting the plant against death by freezing through their ability to hold water, must not be lost sight of, and the power of the colloidal complex in the protoplasm of the plant sap and cell walls in reabsorbing the moisture which is lost during the freezing process, cannot be disregarded. Although entirely hypothetical, is it not possible to assume that in the above experiment, water was removed from the plant cells in the freezing process and that this water could have been reabsorbed by the colloidal complex if these plants had thawed slowly? This assumption takes for granted that the precipitation of the plant proteins assumed to occur at a temperature of – 20° F. was reversible and that the plant would have recovered from the injurious effects of the cold, if it had been placed in an environment favorable for reversal. Such a favorable condition would account for the recovery of the plants which remained outdoors until spring. The plants which were taken into the greenhouse rapidly lost the water removed from the cells in freezing, which, with the transpiration from the leaves, left the plant in a desiccated condition, since no more water could be taken up from the frozen soil. Such a condition would tend to produce an irreversible state in the precipitated protein due to drying, which would not allow rehydration even when moisture became available.

The above is merely suggestive and certainly needs to be substantiated. The plant behavior as previously described is well established and it remains to correlate this behavior with known chemical phenomena. Chemical analyses have demonstrated that impure proteins are precipitated to a greater or lesser extent by freezing, and that carbohydrate compounds act as a protection against protein precipitation, or possibly function in the retention of water. It remains to be determined how these compounds act in the plant and how their physiological interchanges affect cold hardiness.

Physical analyses of sap expressed from plants at various stages of development

Osmotic pressure

Recent investigations on winter hardiness have centered primarily around factors which are correlated with the ability of plants to resist low temperatures, with an attempt to obtain some specific index which might be employed in determining the hardiness of any given plant. These investi-

gations on the indices of hardiness, whether determined by physical or chemical analyses, deal largely with those factors influencing the water relationship of the plant. The consensus of opinion seems to be that any factor capable of retaining the water in the tissue of the plant, tends directly to an increased hardened condition of the plant. ROSA (**18**) considers the water retaining power as dependent upon the two forces, osmotic pressure and imbibition. These forces may be influenced by other factors. The correlation of the depression of the freezing point of sap with cold resistance seems to be one of the oldest means of attack upon the problems of winter hardiness. LINDLEY (**11**), CHANDLER (**1**), OHLWEILER (**16**), and HARRIS and POPENOE (**6**) obtained positive correlations between sap concentration and hardiness. PANTANELLI (**17**), LEWIS and TUTTLE (**9**), SALMON and FLEMMING (**20**) and NEWTON (**14**) concluded that the depression of the freezing point is not an index to hardiness. In a more recent publication, NEWTON (**15**) concludes definitely that the depression of the freezing point is not a true criterion for judging the hardiness of the plant.

A series of sap concentration measurements was made by the writer during the winter seasons of 1923, 1924, and 1925. The object of this investigation was to determine if osmotic concentration measurements as deter-

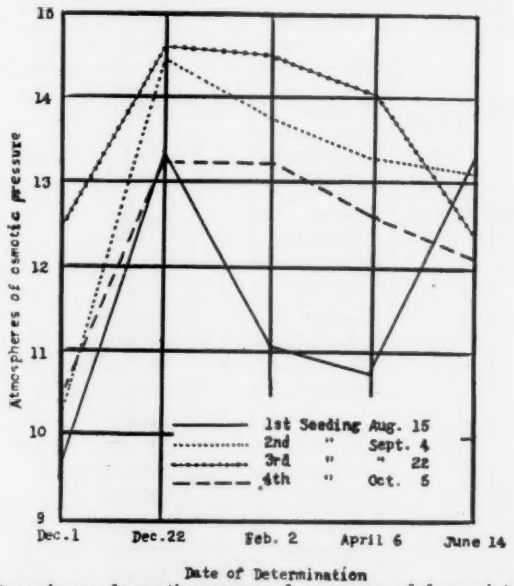

FIG. 3. Atmospheres of osmotic pressure of sap expressed from winter wheat plants at various intervals during the fall and winter months of 1923–1924. Plant collections made from four dates of seeding.

mined by the depression of the freezing point could in any way be correlated with the resistance of the winter wheat plant to cold. A series of measurements was made at intervals during the fall and winter season of 1922, 1923, and 1924, on sap extracted from plants of different dates of seeding. The data are presented in table II and fig. 3. The osmotic pressure of the sap of the wheat plant varies considerably, depending on the stage of development of the plant. In general it may be stated that for the fall and winter of 1923–1924 there was first a rise in osmotic pressure in the sap of all the plants collected up to December 22, after which it decreased gradually until the end of the dormant period, or on the termination of winter. Fig. 3 shows a distinct difference in the osmotic pressure of sap from plants grown from the seedings on different dates. Expressed sap of plants from the third date of seeding had the greatest osmotic pressure throughout the winter,

TABLE II

OSMOTIC PRESSURE, AS DETERMINED BY THE FREEZING POINT DEPRESSION OF SAP EXTRACTED FROM THE WINTER WHEAT PLANTS IN A SERIES OF ANALYSES MADE DURING THE FALL AND WINTER MONTHS OF 1923–1924

ANALYSES MADE ON PLANTS FROM FOUR DATES OF SEEDING

DATE COLLECTED	DATE OF SEEDING	OSMOTIC PRESSURE	FREEZING POINT DEPRESSION
		atmospheres	*degrees C.*
December 1	August 15	9.875	0.820
	September 4	10.120	0.850
	September 22	12.410	1.030
	October 4	10.360	0.860
December 22	August 15	13.400	1.125
	September 4	14.500	1.215
	September 22	14.680	1.220
	October 4	13.380	1.120
February 2	August 15	11.090	0.930
	September 4	13.840	1.150
	September 22	14.560	1.210
	October 4	13.360	1.110
April 6	August 15	10.800	0.980
	September 4	13.360	1.110
	September 22	14.080	1.170
	October 4	12.640	1.050
June 14	August 15	13.340	1.100
	September 4	13.100	1.090
	September 22	12.490	1.030
	October 4	12.160	1.020

followed closely in order by the second, first, and fourth seedings. These variations seem to be distinct, especially during the dormant period.

Results on the osmotic concentration of the wheat plant sap from plantings made in 1924 and 1925 are presented in table III. These data indicate the same general rise and fall in sap concentration as in 1923. The data, moreover, are too meager to permit conclusions for this year. In the main there is no correlation with the previous year's results. In 1923 there was

TABLE III

RANGE OF OSMOTIC PRESSURE AS DETERMINED BY DEPRESSION OF THE FREEZING POINT OF SAP EXPRESSED FROM SEEDLING WHEAT PLANTS COLLECTED FROM THE FOUR DATES OF SEEDING MADE DURING THE FALL OF 1924

DATE COLLECTED	DATE OF SEEDING			
	August 22	September 3	September 15	October 3
	atmospheres	*atmospheres*	*atmospheres*	*atmospheres*
September 14	8.817			
September 30	8.877			
November 6	13.840	17.80	9.25	5.844
December 8	20.440	21.52	19.24	17.320

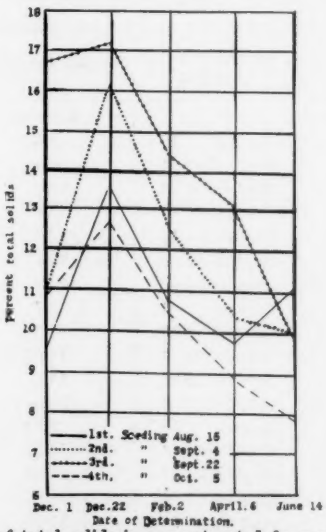

FIG. 4. Percentage of total solids in sap extracted from winter wheat plants, taken from four dates of seeding test plats at various intervals as designated. Dry matter determined with the Abbé refractometer.

a distinct correlation between sap concentration and degree of winter hardiness (see fig. 1 on winter killing).

Not in every instance were chemical analyses made on sap used in measuring the freezing point depression. No doubt such analyses would have revealed some interesting facts regarding the actual substances responsible for the depression of the freezing point. To gain some knowledge of the concentration of the sap, however, the total solids were determined. This was done with the aid of the Abbé refractometer, as described by GORTNER (5); but instead of using the sugar scale for the dry-matter reading, the total solids were obtained from Gerrling's table used in determining the total solids in sugar house products and given in the Methods of the Association of Official Agricultural Chemists. This method was checked by drying a known quantity of sap in the oven at a temperature of 70° C. A comparison of the results is given in table IV. The differences seem to fall within the range of experimental error.

TABLE IV

PERCENTAGE OF TOTAL SOLIDS IN SAP EXTRACTED FROM WINTER WHEAT PLANTS, TAKEN FROM FOUR DATES OF SEEDING ON THE TEST PLATS, AT VARIOUS INTERVALS DURING THE FALL AND WINTER OF 1923 AND 1924

RESULTS ARE GIVEN OF TOTAL SOLIDS WHEN DETERMINED WITH THE ABBÉ REFRACTOMETER, AND WHEN SAP WAS EVAPORATED IN THE OVEN AT 65°–70° C.

DATE COLLECTED	DATE OF SEEDING	REFRACTOMETER	OVEN AT 65°–70° C.
		per cent.	*per cent.*
December 1	August 15	9.6	9.50
	September 4	10.9	10.26
	September 22	16.8	16.70
	October 4	10.9	10.47
December 22	August 15	13.6	11.69
	September 4	16.1	14.87
	September 22	17.2	16.51
	October 4	12.7	13.28
February 2	August 15	10.8	10.197
	September 4	12.65	12.393
	September 22	14.5	14.000
	October 4	10.6	10.200
April 6	August 15	9.70	9.60
	September 4	10.45	10.00
	September 22	13.20	13.10
	October 4	8.90	8.40
June 14	August 15	11.05	11.20
	September 4	10.00	10.20
	September 22	9.80	8.10
	October 4	6.85	6.45

The data for total solids in the sap of plants from various dates-of-seeding for 1922–1923 are plotted in fig. 4. It will be noted that the total sap solids vary directly with the osmotic pressure as indicated in fig. 3. The total solids increased up to December 22, and then decreased until the opening of spring, April 6.

TABLE V

PERCENTAGE OF TOTAL SOLIDS IN SAP EXTRACTED FROM WINTER WHEAT PLANTS, TAKEN FROM FOUR DATE-OF-SEEDING TEST PLATS, AT VARIOUS INTERVALS DURING THE FALL OF 1924

DATE OF COLLECTION	DATE OF SEEDING			
	August 22	September 3	September 14	October 3
	per cent.	*per cent.*	*per cent.*	*per cent.*
September 14	3.3	.....	.....	.....
September 30	2.8	.....	.....	.....
November 8	7.2	7.6	5.6	3.60
December 8	7.6	8.2	7.4	5.75

The 1924 results for sap solids parallel the 1924 data on osmotic pressure. There is a progressive increase in both total sap solids and osmotic pressure as the season advances. The greatest osmotic pressure was obtained on December 8 from plants of the second date-of-seeding, followed closely by the plants of the first, third, and fourth seedings. The 1924 osmotic pressure data presented in table III, however, show no correlation with similar data obtained in the fall of 1923. In the latter year, as noted previously, the sap concentration was greatest in plants from the third seeding followed in order by that in plants from the second, first, and fourth date of seeding. These discrepancies may be due to the difficulty of obtaining complete extraction from young plant tissues. Total sap solids seem to be positively correlated with the relative winter hardiness of the plants from the various dates of seeding for the year 1923, but no correlation exists for the year 1924.

Chemical composition of sap

Data on the chemical composition of sap extracted in the manner described under methods are presented in table VI. The composition of the residue is also given. The object of this experiment was to determine quantitatively the constituents of the expressed sap which was used for the determination of osmotic pressure and total solids. The analyses of the carbohydrate and nitrogenous compounds of sap extracted from plants from the

plots sown on the first date were made on October 3, 1924. It will be noted that 0.78 per cent. nitrogen, or 27.86 per cent. of the total nitrogen, was recovered in the extracted sap. Table VII gives data showing the sap composition of plants from the four dates of seeding. These data indicate that a greater proportion of both nitrogenous and carbohydrate substances are recovered in the sap of plants from the early dates of seeding. The percentage of total nitrogen in the sap ranges from 85 per cent. in plants from the first seeding, to 35.25 per cent. in plants of the fourth seeding. Similarly, the percentage of the total sugars extracted with the sap, ranges from 100 per cent. in the plants from the first seeding to 50.8 per cent. in the plants from the fourth seeding. The residue retained a greater proportion of both carbohydrate and nitrogenous compounds in young plants. Whether more of these compounds are tied up in structural materials in the younger plants, or whether the larger proportion of embryonic tissue and the smaller amount of vacuolar material in the young plant is responsible for the less complete extraction of sap in the latter, cannot be stated. The data do indicate, however, that it is the free and available substances released through grinding and extraction that determine the osmotic pressure and total solids in the plant sap, and that these readily available substances differ in character and quantity for the different dates of seeding. Thus while the data on osmotic pressure and total solids obtained in 1923 to 1924 correlate with the relative hardiness of plants from the various dates of seeding for that year (see fig. 1), such correlation is not noted in data obtained for 1924 to 1925. The lack of such correlation is no doubt due to

TABLE VI

CHEMICAL COMPOSITION (CARBOHYDRATE AND NITROGEN) OF SAP EXPRESSED FROM THE CROWNS OF WINTER WHEAT PLANTS AND THE RESIDUE OF PLANTS FROM DIFFERENT DATES OF SEEDING

COMPOSITION OF SAP		COMPOSITION OF RESIDUE	PERCENTAGE REMOVED THROUGH EXTRACTION
	per cent.	*per cent.*	*per cent.*
Total nitrogen	0.78	2.02	27.86
Protein nitrogen	0.35	.....	.....
Filtrate nitrogen	0.43	.....	.....
Total sugars	8.86	2.80	75.90
Reducing sugars	5.54	1.17	.....
Sucrose	2.18	0.40	.....
Dextrins	0.846	0.95	.....
Starch	0.0	0.35	.....
Hemicellulose	0.0	15.80	.....
Soluble carbohydrate	9.706	.....	32.84

several factors, among which may be mentioned first, a lack of correlation of hardiness with the soluble carbohydrate and nitrogenous compounds in the plant at different stages of growth, and secondly the difference in degree

TABLE VII

CHEMICAL COMPOSITION (CARBOHYDRATE AND NITROGEN) OF SAP EXPRESSED FROM THE CROWNS OF WINTER WHEAT SEEDLINGS, AND OF THE RESIDUE OF PLANTS FROM DIFFERENT DATES OF SEEDING

Composition	Sap				Residue			
	Date of seeding				Date of seeding			
	Aug. 22	Sept. 4	Sept. 14	Oct. 3	Aug. 22	Sept. 4	Sept. 14	Oct. 3
	per cent.	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*	*per cent.*
Total nitrogen	1.70	1.50	1.30	1.10	0.30	1.13	1.57	2.02
Reducing sugar	9.20	6.86	4.96	5.70				
Total sugar	14.52	13.20	8.64	7.56	0.00	1.50	5.20	7.20
Dextrins					0.00	2.00	0.50	1.00
Starch					0.00	0.00	0.00	0.00
Hemicellulose					15.00	16.50	15.30	12.00

in which the plant tissue releases such soluble compounds with the ordinary methods used in the extraction of the sap. The chemical study in relation to winter killing is discussed in detail in another paper (7).

Summary

1. The percentages of winter killing of winter wheat plants, determined on date-of-seeding plats during the three years 1923 to 1925, inclusive, were found to vary with the date of seeding. The seedings were made on or close to the following dates, namely, August 15, August 31, September 21, October 6, and October 19. The average winter killing in the three years was found to be greatest in the October 19 seeding, followed in order by those of October 6, August 31, and September 21, respectively.

2. Freezing and rate-of-thawing tests on plants taken from the date-of-seeding plats show that sudden changes of temperature within a large temperature range affect the subsequent recovery of the plant. The temperature at which the plant grows, previous to freezing, is important from the standpoint of the recovery of the plant after freezing. Plants grown at high temperatures (33° C.) were killed when subjected to a freezing temperature of −10° C., whereas plants grown at a temperature of 5° C. were not seriously injured when subjected to −10° C. Rapid thawing of plants

which had been frozen at – 20° C. resulted in their death. Those frozen at a temperature of – 5° C. showed no serious injury when subjected to the same rapid rate of thawing.

3. In a series of osmotic pressure determinations of sap made during 1923 and 1924 on plants from the various dates of seeding, it was found that the quantity of sugars and dextrins in the sap determined the osmotic pressure; but the relative quantities of these substances taken out of the plant tissues with the extracted sap varied with the different dates of seeding. It is concluded, therefore, that the osmotic pressure measurements of sap from wheat plants at various stages of development do not indicate the degree of winter hardiness of the plants.

The writer wishes to express his appreciation to Dr. E. J. KRAUS, Dr. JAMES DICKSON, and Prof. B. D. LEITH for their helpful suggestions and criticisms given during this investigation.

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LITERATURE CITED

1. CHANDLER, W. H. The killing of plant tissue by low temperature. Missouri Agr. Exp. Sta. Res. Bull. 8. 1913.
2. GARDNER, V. R., BRADFORD, F. C., and HOOKER, JR., H. D. Fundamentals of fruit production. McGraw-Hill Co. (See pp. 261–262.)
3. GORKE, H. Über chemische Vorgänge beim Erfrieren der Pflanzen. Landw. Vers. Stat. **65**: 149–160. 1907.
4. GORTNER, R. A., and HARRIS, J. A. Notes on the technique of the determination of the depression of the freezing point of vegetable saps. Plant World **17**: 49–53. 1914.
5. ————, and HOFFMAN, W. Determination of moisture content of expressed plant tissue fluids. Bot. Gaz. **74**: 308–313. 1922.
6. HARRIS, J. A., and POPENOE, W. Freezing-point lowering of the leaf sap of the horticultural types of *Persea americana*. Jour. Agr. Res. **7**: 261–268. 1916.
7. JANSSEN, GEO. Effect of date of seeding of winter wheat upon some physiological changes of the plant during the winter season. Jour. Amer. Soc. Agron. **21**: 168–200. 1929.
8. ————. Effect of date of seeding of winter wheat on plant development and its relationship to winter hardiness. Jour. Amer. Soc. Agron. **21**: 444–466. 1929.
9. LEWIS, F. J., and TUTTLE, G. M. Osmotic properties of some plant cells at low temperatures. Ann. Bot. **34**: 405–416. 1920.

10. LIDFORSS, B. Zur Physiologie und Biologie der wintergrünen Flora. Botan. Centralbl. **68**: 33–44. 1896.
11. LINDLEY, J. Philosophy of the destruction of plants by frost. Trans. London Hort. Soc. Ser. 2, Vol. 2, Part IV. Reprinted in Horticulturist **7**: 405–411. 1852.
12. MOLISCH, HANS. Untersuchungen über das Erfrieren der Pflanzen. 1897.
13. MÜLLER-THURGAU, H. Ueber das Gefrieren und Erfrieren der Pflanzen. Landw. Jahrb. **9**: 133–189. 1880.
14. NEWTON, ROBERT. A comparative study of winter wheat varieties with especial reference to winter-killing. Jour. Agr. Sci. **12**: 1–19. 1922.
15. ————————. The nature and practical measurement of frost resistance in winter wheat. Alberta Agr. Exp. Sta. Res. Bull. 1. 1924.
16. OHLWEILER, W. W. The relation between the density of cell saps and the freezing points of leaves. Ann. Rept. Missouri Bot. Gard. **23**: 101–131. 1912.
17. PANTANELLI, E. Sul la resistenza delle plante al freddo. Atti. R. Acad. Lincei. **27**: 126–130, 148–153. 1918.
18. ROSA, J. T., JR. Investigations on the hardening process in vegetable plants. Missouri Agr. Exp. Sta. Res. Bull. 48. 1921.
19. SACHS, J. Krystallbildungen bie dem Gefrieren und Veränderung der Zellhäute bei dem Aufthauen saftiger Pflanzentheile. Ber. Ver. Kön. Sachs. Gesell. Wiss. zu Leipzig **12**: 1–50. 1860. See also Landw. Ver. Stat. **2**: 167–201. 1860.
20. SALMON, S. C., and FLEMING, F. L. Relation of the density of cell sap to winter hardiness in small grains. Jour. Agr. Res. **13**: 497–506. 1918.
21. SCHANDER, R., and SCHAFFNIT, E. Untersuchungen über das Auswintern des Getreides. Landw. Jahrb. **52**: 1–66. 1918.
22. WEST, F. L., and EDLEFSEN, N. E. The freezing of fruit buds. Utah Agr. Exp. Sta. Bull. 151. 1917.
23. WIEGAND, K. M. Some studies regarding the biology of buds and twigs in winter. Bot. Gaz. **41**: 373–424. 1906.

SOME EFFECTS OF CALCIUM DEFICIENCY ON *PISUM SATIVUM*

DOROTHY DAY

(WITH FIVE FIGURES)

Introduction

It has long been known that the addition of calcium to the soil has a beneficial effect in the growing of crops. PLINY described the chalk wells from which chalk was brought up to be spread over the surface of the soil of southern England where the land is deficient in lime. Many agricultural bulletins emphasize the need of this element, especially in growing leguminous crops.

In general, the external appearance of the plant has been employed as a standard of comparison by many investigators who have considered the effect on the plant of many variations in the conditions of growth, including the omission of certain salts from the nutrient solution.

The literature covering the necessity of calcium for the normal growth of plants and animals is vast. S'JACOB (**4**) finds that a deficiency of calcium produces similar results in both peas and beans. He describes the plants as somewhat limp and yellowish, with roots little branched, and often slimy by the end of the experiment. A recent investigation by CARGILL (**1**) shows that peas grown in a complete nutrient solution vary markedly in appearance if the ratio of calcium to magnesium is altered. In a measure her study has furnished the basis of the present investigation.

More recently workers have investigated the variations caused in the internal structure by alterations of external conditions. Before much can be accomplished in this line, it is necessary to study the structure of a normal plant, as has been done by COMPTON (**2**), who discusses the seedling structure of the Leguminosae. COMPTON gives a fairly clear picture of the arrangement of the principal tissues in many of the important forms, including the pea.

SOROKIN and SOMMER (**8**) state that the absence of calcium causes marked and rapid changes in the root tips of *Pisum sativum*, variety Golden Vine. They conclude that this element is a constituent of the protoplast essential for normal mitotic division in the meristematic region. Their evidence seems to show that a lack of calcium does not cause disintegration of tissues by separation of the cells through the absence of calcium pectate as has been suggested by some writers.

One general conception has been that calcium is important in neutralizing the acid in the soil. Doubtless this is not its only function. It has been the aim of this study to determine the effect of calcium, not with

reference to its rôle in the soil nor at the absorbing surfaces of the plant, but as a factor influencing the anatomical structure of the plant.

Materials and methods

In this investigation Canada field peas (*Pisum sativum* L.) were grown in sand cultures in the greenhouse. Fine white Ottawa quartz sand known as "general run," which RIKER (**6**) had previously determined to be lacking in available essential elements, was used. Earthenware jars, glazed to prevent leaching, were employed. All plants were subject to the same variations in the conditions of the greenhouse, such as light, temperature, and relative humidity.

SERIES I

In the preliminary series the composition of the nutrient solution was based upon that used by CARGILL (**1**) in growing peas of the same variety. Solutions of the several salts in distilled water were made up in liter quantities to serve as stock solutions. These were kept in Pyrex glass flasks tightly stoppered with corks wrapped in tinfoil. From these were compounded the three culture solutions which varied only in their content of calcium nitrate. For the complete nutrient solution (A) there was used the proportion which CARGILL (**1**) found to produce the greatest weight and the second greatest length for similar peas. Solution B contained one-half the amount of calcium nitrate present in solution A, while solution C lacked it entirely. In each case distilled water was added to make 1000 cc.

TABLE I

CULTURE SOLUTIONS USED IN SERIES I

SALTS	CULTURE SOLUTIONS, GRAM PER LITER		
	FULL NUTRIENT SOLUTION	SOLUTION MINUS ONE-HALF CALCIUM	SOLUTION MINUS CALCIUM
	A	B	C
	gm.	*gm.*	*gm.*
KNO_3	0.2022	0.2022	0.2022
KH_2PO_4	0.2723	0.2723	0.2723
K_2SO_4	0.3485	0.3485	0.3485
$FeCl_3 \cdot 6H_2O$	0.0189	0.0189	0.0189
$Mg(NO_3)_2 \cdot 6H_2O$	0.9115	0.9115	0.9115
$Ca(NO_3)_2 \cdot 4H_2O$	0.4641	0.2321	0.0

On April 3, 1926, four seeds were placed in each of the jars containing sand moistened with the nutrient solution to be tested. It was the aim at all times to keep the sand well moistened with the respective nutrient solution, but an excess was avoided. After two weeks the two most vigorous

plants in each pot were allowed to remain while the other two were removed. Supports were inserted in the sand and the plants were tied to them. Beginning at this time and thereafter once each week, the sand at the top was flushed in the evening with distilled water which drained at the bottom through a hole uncorked for that purpose. This was repeated immediately and the jars were allowed to drain over night. The next morning the corks were replaced and enough nutrient solution was added to moisten but not to saturate the sand.

After five weeks one-third of the pots containing solution C (four plants) were discarded because of the death of the plants. The remaining pots were numbered consecutively. From this time on the complete nutrient solution (A) was added to one-half the pots (four plants). The others were treated as before. This was continued until all plants were harvested on June 14, 1926, at the end of ten weeks of growth. The pots containing solutions A and B were treated as before.

At the time of harvest observations were made as to the external aspects of stems and roots. Definite portions of stems, roots, and leaves from each plant were fixed in formalin-acetic-alcohol, embedded in parowax, sectioned, and stained. These sections were compared with similar sections of roots and stems grown in the soil under the usual garden conditions.

Series II

In the second series one-tenth molar solutions of the several salts in distilled water were made up in liter quantities to serve as stock solutions. Sodium nitrate was used in addition to these salts employed in series I. From these were compounded the seven culture solutions as shown in the following table. In each case distilled water was added to make 1,000 cc.

TABLE II

Culture solutions used in series II

Salts	Culture solutions, gram per liter						
	Full nutrient solution	Solutions minus one-half calcium			Solutions minus calcium		
	A	B	D	F	C	E	G
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
KNO_3	0.2022	0.2022	0.2528	0.2780	0.2022	0.3033	0.3539
KH_2PO_4	0.2723	0.2723	0.2723	0.2723	0.2723	0.2723	0.2723
K_2SO_4	0.3485	0.3485	0.3485	0.3485	0.3485	0.3485	0.3485
$FeCl_3 \cdot 6H_2O$	0.0135	0.0135	0.0135	0.0135	0.0135	0.0135	0.0135
$Mg(NO_3)_2 \cdot 6H_2O$	0.6413	0.6413	0.6413	0.6733	0.6413	0.6413	0.7054
$Ca(NO_3)_2 \cdot 6H_2O$	0.2362	0.1181	0.1181	0.1181	0.0	0.0	0.0
$NaNO_3$	0.0	0.0	0.4250	0.0	0.0	0.8500	0.0

In solutions A, B, and C the only difference is in the amount of calcium nitrate with the proportions of A to B to C, the same as in the earlier series, although the amount of calcium nitrate in each solution has been decreased. In solutions D and E the same ratio holds, but the nitrate content is compensated in chemically equivalent amounts by equal parts of sodium nitrate and potassium nitrate. In solutions F and G there is a similar substitution of the nitrate, but one-fourth of the nitrate was replaced by magnesium nitrate and three-fourth by potassium nitrate; therefore there is a substitution for the nitrate radical rather than a molecular substitution.

The peas were first sterilized in a weak formalin solution (1 part in 250), and rinsed in distilled water. The pots were washed in one and one-half per cent. formalin and rinsed in distilled water. On February 8, 1927, five seeds were placed in each of the jars containing sand moistened with the nutrient solution. Six jars were used for each solution. After two weeks all plants in each pot were removed except the two most vigorous ones. Supports were inserted for each plant. Beginning at this time the pots were flushed with distilled water and refilled with the nutrient solution as in the preliminary series.

At the end of five weeks' period of growth, observations were recorded as to the general appearance and height; the measurement was from the cotyledons to the top of the plant as it was growing. The plants were harvested as in the preceding series except that only one from each pot was fixed. This material was treated as in the earlier series. For the second plant green weight and dry weight determinations were made for shoots and for roots, and the material was saved for calcium analyses.

Prepared sections of roots and stems were studied microscopically and were compared as in the preceding series. In addition, the actual area of each of several tissues in every cross section of a stem or root was calculated.

Results

External appearance of plants

Series I.—A difference in the plants of the preliminary series became noticeable soon after the second week of growth and very marked by the end of five weeks. The plants starved of calcium (C) were much smaller and more chlorotic. One-third of these plants (four) had died. The twelve plants treated with solution B were taller in general than the twelve given the complete nutrient solution (A).

At the end of ten weeks the four plants starved of calcium for this entire period showed effects of the lack of this element even more than previously; in fact, about one-half of them died before this time. Those

to which the complete nutrient solution was added during the last five weeks of growth showed distinct recovery (fig. 1). Two of these elongated beyond some of the plants treated with the complete nutrient solution

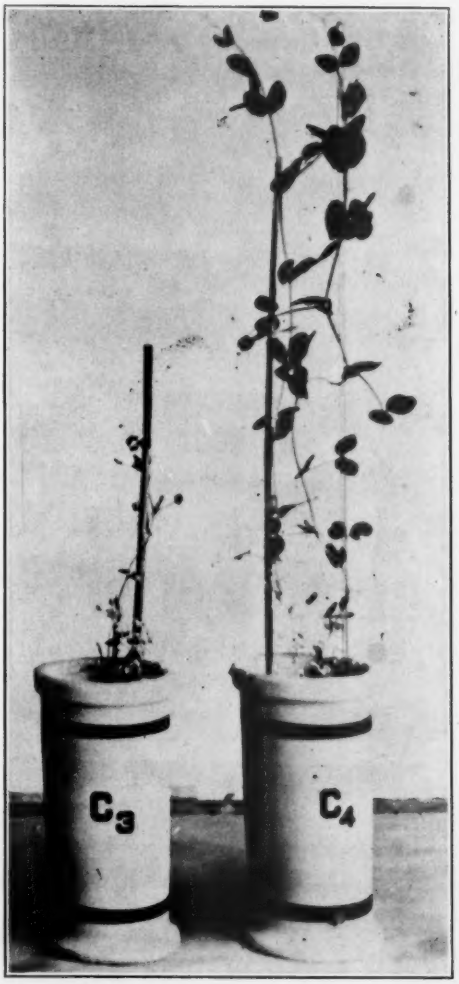

FIG. 1. Plants of series I starved of calcium for the first five weeks. Pot C3 received no calcium for five subsequent weeks while pot C4 was treated with the complete nutrient solution.

throughout the entire period. It is impossible to say how far plants may be starved of calcium and yet be restored when this element is supplied.

In general, those plants given solution B were taller than those given solution A and seemed fully as vigorous. Flowers were borne on two plants only, these in solution B.

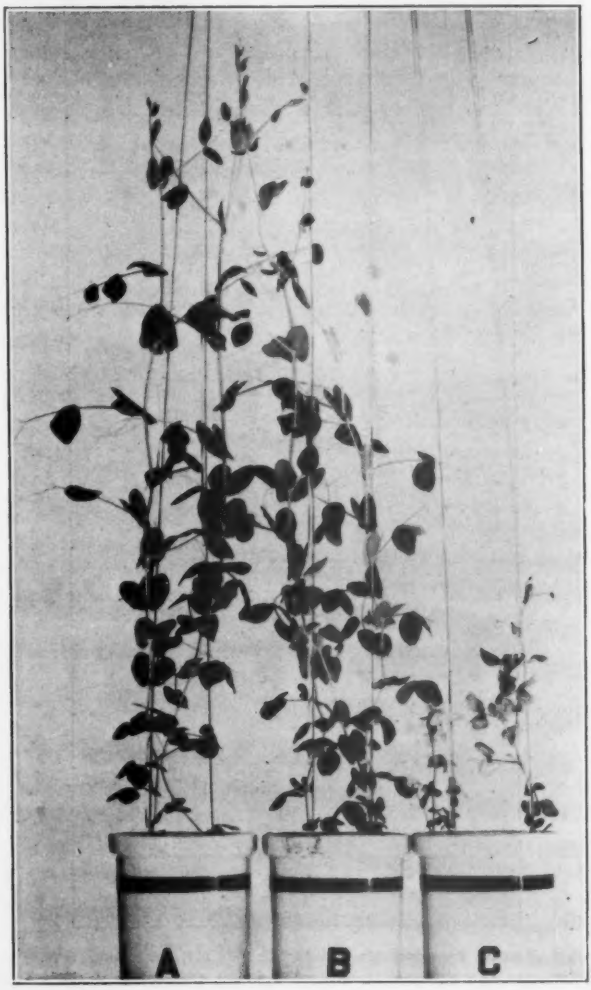

FIG. 2. Plants of series II grown in full nutrient solution (A), in a solution minus one-half the amount of calcium (B), and in a calcium-free solution (C).

SERIES II.—As in the plants of the preliminary series, a difference became noticeable soon after the second week of growth and was still more evident at the end of five weeks (fig. 2). In general, the twelve plants with the complete solution (A) seemed to be fairly uniform in height with an average of twenty-eight inches above the cotyledons. Those given the solutions with half the amount of calcium (B, D, F) showed little variation among the three groups, and were only one inch (B, D) or three inches

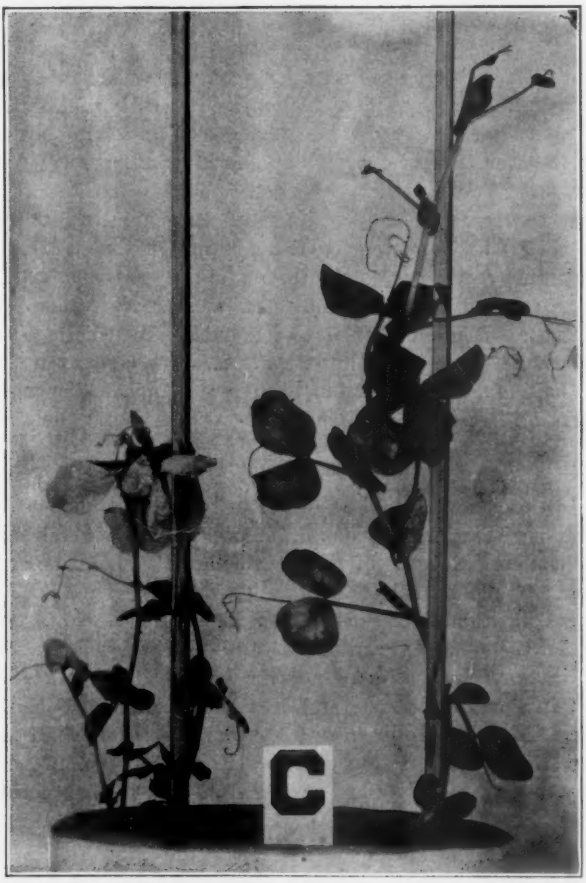

FIG. 3. Plants grown in a calcium-free solution (C). The lower leaflets and stipules show the characteristic chlorosis. In one plant the curling of the upper leaves is evident.

(F) shorter than those with the complete nutrient solution (A). There was, however, a decided difference between any of these and the plants deprived of calcium (C, E, G) although the latter showed little variation within the group as a whole. These plants were about one-half as tall as those given the complete nutrient solution (A).

The plants deprived of calcium exhibited definite changes in the leaves. The lower leaflets and stipules were chlorotic; the portions around the edge of the stipule, or leaflet, and at the base of the main vein, remained fairly green, although yellowish, while the part between these became a creamy white. The uppermost stipules and leaflets were decidely curled and rigid almost to the point of a leathery toughness. These characteristics are best shown in a photograph of a plant treated with solution C, although all these plants were very similar (fig. 3). The plants given the half portion of calcium showed a very slight amount of curling; few or none were chlorotic.

The roots were remarkably uniform as to general appearance, being well developed with numerous secondary roots. None of them showed the short, stubby primary roots with few knobby laterals, often the case when either calcium or magnesium is in excess. No attempt was made to record the exact number or size of nodules. In general, those plants given the complete nutrient solution have more and larger nodules, those given the half amount of calcium have fewer and smaller nodules, and those deprived of calcium have no nodules, or a few extremely small ones.

Anatomical structure

Root.—In each series, sections of the primary roots cut one inch below the cotyledons showed no obvious differences in the general structure or in the proportional amounts of tissues, whether the plants were given the full amount of calcium, half that quantity, or none (fig. 4). A study of the averages (arithmetic means) given in table III shows that in the second series the plants grown in the full nutrient solution (A), when measured in cross section, were not the largest in total area, although the largest in area of the stele and area of the xylem. When these are compared in these three respects with the plants given one-half the amount of calcium (B, D, F), it is seen that the latter rank first in one case only (total area for B), but they are second from every other point of view. The plants starved of calcium (C, E, G) are the smallest in all three measurements.

If these same regions are compared to find the relation of area of stele to total area, the largest ratio is in the plants starved of calcium (E), while second are the plants given the complete nutrient solution (A). Comparisons of area of xylem to total area and of area of xylem to area

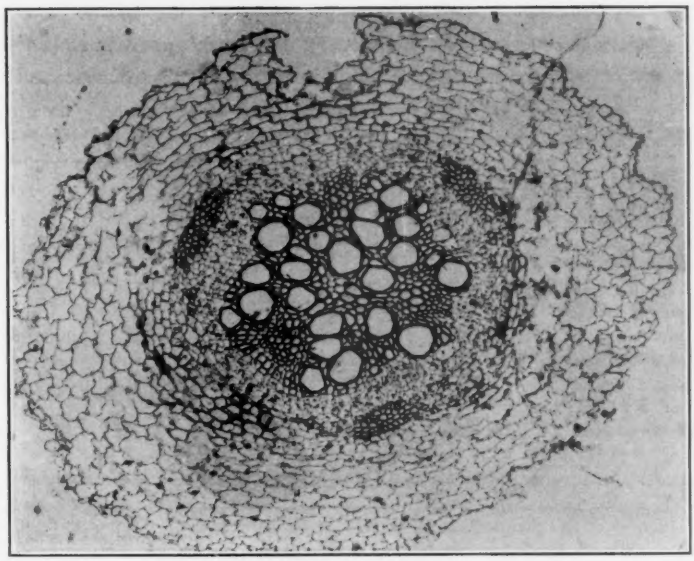

FIG. 4. Cross-section of a root cut one inch below the cotyledons of a plant starved of calcium (G). Magnification 88.

of stele show that in each case the ratio is greatest in the plants treated with the full nutrient solution (A). The plants given half the amount of calcium (B, D, F) do not show regularly a larger proportion of xylem than do those plants starved of calcium (C, E, G).

No distinct variations are noted when other sections of roots are studied in a similar but less detailed manner, although observations have been made at several levels for each root of each series. In all cases there is practically no anatomical difference between these plants and those grown under the usual garden conditions. As will appear later, the same holds true for the stem.

STEM.—In each series, sections of the main stem cut in the middle of the third internode show no striking differences as to the general structure or proportional amounts of tissues, regardless of the calcium treatment of the plant concerned (fig. 5). The averages given in table IV show that in the second series the plants in the full nutrient solution (A), when measured in cross section, are not the largest in total area, but they are largest in area of stele, and area of xylem. This was also true in the case of the root, but, for the stem, the greatest in total area is one group

TABLE III

SECTIONS OF PRIMARY ROOTS OF PEA ONE INCH BELOW THE COTYLEDONS

REGIONS MEASURED	COMPLETE NUTRIENT SOLUTION	SOLUTIONS MINUS ONE-HALF CALCIUM			SOLUTIONS MINUS CALCIUM		
	A	B	D	F	C	E	G
Total area in sq. mm.*	1.4728	1.5354	1.1476	1.1451	0.9252	0.9772	0.9425
Area of stele in sq. mm.*	0.4422	0.4145	0.3399	0.3276	0.2738	0.3009	0.2460
Area of xylem in sq. mm.*	0.2149	0.1853	0.1553	0.1478	0.1213	0.1322	0.1105
Area of stele: total area	0.3002	0.2700	0.2962	0.2861	0.2959	0.3079	0.2610
Area of xylem: total area	0.1459	0.1207	0.1353	0.1291	0.1311	0.1353	0.1172
Area of xylem: area of stele	0.4860	0.4470	0.4569	0.4512	0.4430	0.4393	0.4492

* Average of five plants from series II.

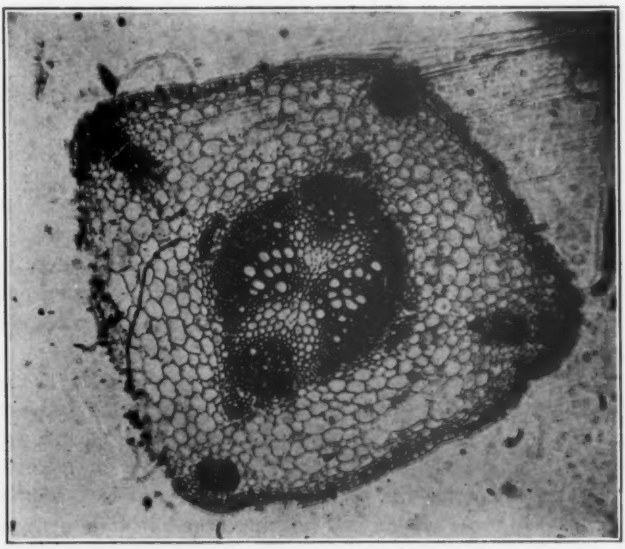

FIG. 5. Cross-section of a stem cut in the third internode of a plant starved of calcium (E). Magnification 39.

of plants starved of calcium (E), while the same plants rank second as to area of stele and area of xylem. For these three measurements no general statement can be made except that in total cross section area the plants deprived of calcium are usually larger than the plants given the half portion of calcium while the reverse is true for the area of stele and area of xylem.

If these same regions be compared to find the relation of area of stele to total area, of area of xylem to total area, and of area of xylem to area of stele, it may be seen from table IV that in each case the largest ratio is found in the plants grown in the full nutrient solution (A), while those given the half calcium (B, D, F) are second, and those starved of calcium (C, E, G) are third.

For the points just discussed, the stem and root do not follow the same variations to any marked extent.

A study of sections of the main axes at higher internodes offers no striking differences in either series. A comparison of sections taken in the fifteenth internode was attempted but the study could not be made, as some plants did not elongate to that extent. In general the number of internodes is proportional to the amount of calcium in the nutrient solution with a variation from eighteen internodes in the plants provided with the most calcium, to fourteen in those starved of calcium.

TABLE IV

SECTIONS OF STEMS OF PEA TAKEN IN THE THIRD INTERNODE

REGIONS MEASURED	COMPLETE NUTRIENT SOLUTION	SOLUTIONS MINUS ONE-HALF CALCIUM			SOLUTIONS MINUS CALCIUM		
	A	B	D	F	C	E	G
Total area in sq. mm.*	2.4915	2.2706	2.3633	2.2550	2.2777	2.5229	2.3887
Area of stele in sq. mm.*	0.6159	0.5413	0.5573	0.5369	0.4978	0.5795	0.4992
Area of xylem in sq. mm.*	0.2306	0.1934	0.1922	0.1954	0.1597	0.1981	0.1577
Area of stele: total area	0.2472	0.2384	0.2358	0.2381	0.2186	0.2297	0.2090
Area of xylem: total area	0.0926	0.0852	0.0813	0.0867	0.0701	0.0785	0.0660
Area of xylem: area of stele	0.3744	0.3573	0.3449	0.3639	0.3208	0.3418	0.3159

* Average of five plants from series II.

Discussion

In each series there is found the customary difference in elongation since the total length is greater and there are more internodes when calcium is present in the nutrient solution. As described by S'JACOB (**4**) the lower leaves of calcium starved plants "become yellow and look as if they were first sucked out by the upper leaves." Contrary to his description of the plant as limp, it has been found here that the upper leaves are stiff almost to the point of a leathery toughness. S'JACOB makes no mention of the curling of the upper leaflets and stipules. That these results seem to be due to lack of calcium is shown by those calcium-starved plants which resumed normal growth as soon as the complete nutrient solution was added.

The greater uniformity of growth and the production of some flowers by plants which received half the amount of calcium seem to indicate a better proportion of the elements in this combination than in the complete nutrient solutions. KNOP'S solution as given by PALLADIN (**5**) contains 0.571 gram of calcium nitrate per liter for very young plants, and 2.855 grams per liter for older plants. In SHIVE'S solution 1.228 grams of calcium nitrate per liter gave the best results with wheat and 1.842 grams per liter gave the second best (**7**). In this investigation the complete nutrient solution of the first series contained 0.4641 gram of calcium nitrate per liter. As compared with this amount of calcium in the nutrient solution, it is one-half this quantity that appears to give better results based on uniformity of growth of plants and on the production of flowers. Is it possible that the proportion of calcium generally used is too high?

Working with *Phaseolus vulgaris,* S'JACOB (**4**) decided that an excess of this element is possible, although *Pisum sativum* did not show it in his experiments. He stated that the harmful effect is due to an excess of calcium ions rather than to a high concentration of calcium carbonate. This is in disagreement with the conclusions of GILE and CARRERO (**3**). Whether it is purely an effect of an excess of calcium ions is not proven by this investigation, but it seems at least plausible that several nutrient solutions in common use contain more than an optimal amount of calcium.

Because the effects of the absence of calcium appeared so quickly SOROKIN and SOMMER (**8**) found it desirable to germinate pea seeds (*Pisum sativum,* variety Golden Vine) in a solution of calcium sulphate and then transfer the seedlings to the calcium free solutions. This variety seems to be extremely sensitive to the absence of calcium as no such difficulty was encountered in this investigation with *Pisum sativum,* variety Canada field, since all seeds were germinated in the same nutrient solution as that in which they were allowed to continue growth. It is possible that the

latter variety contains a larger amount of stored calcium thus permitting some growth at the first, followed by marked repression later in those cases where calcium was lacking.

Since their plants were grown in water cultures it is hardly possible to compare the resulting structures with those of plants grown in sand cultures. Seedlings in a nutrient solution frequently show abnormalities not evident when similar seeds are grown in pure sand treated with another portion of the same nutrient solution. However, their work agrees with the results noted by the author in the conclusion that absence of calcium does not result in separation of the cell walls. If the material examined in these studies had shown a hindrance of mitotic division such as they observed in their material it would be impossible to distinguish the tissues of normal appearance as previously noted.

In the present investigation a comparison of the anatomical structure of the root as seen in cross section showed no consistent variation whether the plant was grown under the usual garden conditions, in a complete nutrient solution, or in one which entirely lacked calcium. This holds true for sections taken at different levels. A similar comparison of stems showed no greater variation in the tissues. The only conclusion that can be reached on the basis of this investigation is that the lack of calcium causes no outstanding modification in anatomical structure. That the difference appears to be in amount of elongation, rather than in kind of structure, is supported by the measurements of the growing plants which show greater total length and have more internodes if calcium is present in the nutrient solution.

Summary

1. Canada field peas were grown in nutrient solutions in which the proportion of calcium nitrate was varied. Observations were recorded for external appearance and for anatomical structure.

2. These results support the contention that lack of calcium affects the external appearance of plants since plants starved of calcium are shorter, the lower leaves are chlorotic, and the youngest leaves are curled and tough.

3. A comparison of the proportion of calcium used here with that used by KNOP and SHIVE seems to indicate that several nutrient solutions in common use contain more than an optimal amount of this element.

4. Whether the plants are grown under the usual garden conditions, in a nutrient solution containing the full quantity of calcium, or in a solution lacking calcium, the anatomical structure of the root and of the stem remains practically constant.

5. The difference is a variation in the amount of elongation rather than in the anatomical structure as indicated by transverse sections.

This study was begun at the University of Wisconsin where most of the experimental work was completed. I wish to thank Professor J. B. OVERTON for his advice and helpful suggestions during the progress of this investigation. To Professor E. J. Kraus I desire to express my appreciation for criticism of some of the work.

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SMITH COLLEGE.

LITERATURE CITED

1. CARGILL, MIRIAM A. Antagonism between calcium and magnesium in a complete nutrient solution. Unpublished thesis, University of Wisconsin. 1925.
2. COMPTON, ROBERT HAROLD. An investigation of the seedling structure in the Leguminosae. Jour. Linn. Soc. Bot. **41**: 1–122. 1912.
3. GILE, P. L., and CARRERO, J. O. Cause of lime-induced chlorosis and availability of iron in the soil. Jour. Agr. Res. **20**: 33–62. 1920.
4. s'JACOB, J. C. Anorganische beschädigingen bij *Pisum sativum* L. en *Phaseolus vulgaris* L. Doctor's thesis, Utrecht. 1927.
5. LIVINGSTON, B. E. Palladin's plant physiology. 3d Amer. ed. pp. 82–83. Phila. 1926.
6. RIKER, R. S. Studies on the substitution of calcium, sodium, rubidium, and caesium for potassium in the growth of Hubbard squash, White Spine cucumber, and Japanese buckwheat. Unpublished thesis, University of Wisconsin. 1923.
7. SHIVE, J. W. A study of physiological balance in nutrient media. Physiol. Res. **1**: 327–397. 1915.
8. SOROKIN, HELEN, and SOMMER, ANNA L. Changes in the cells and tissues of root tips induced by the absence of calcium. Amer. Jour. Bot. **16**: 23–39. 1929.

THE INSOLUBLE TYROSINASE OF THE VELVET BEAN SEED COAT

EMERSON R. MILLER

The oxidizing enzymes known as tyrosinases are widely distributed in the vegetable kingdom and are also present in invertebrates and in the skin of various animals. They play an essential rôle in the formation of black pigments in the liquids and tissues of vegetable and animal organisms.

Almost all investigations of tyrosinases so far reported concern water-soluble enzymes only. A notable exception was the discovery by GORTNER (**4**) that both a soluble and an insoluble tyrosinase are present in the larvae of the meal worm, *Tenebrio molitor*.

LEHMANN and SANO (**7**) found that *Actinomycetes chromogenes* has the power of converting tyrosine into a dark brown pigment, but they could not extract any tyrosinase from the micro-organism.

The velvet bean now furnishes a very interesting and a very striking example of an insoluble enzyme which is found in the seed coat, and which has the power of oxidizing a chromogen contained only in the cotyledons, in this way producing the color, characteristic of the seeds of certain varieties.

Since this enzyme has the power of oxidizing tyrosine and other compounds containing a hydroxy-phenyl group it is classed with the tyrosinases.

Color formation in the velvet bean

While extracting powdered velvet beans with cold water it was observed that pink colored spots were soon formed throughout the mass. By diffusion, the liquid became uniformly pink and this color gradually changed through various shades of red, brown, purple and finally became black.

On examination of the mass it was found that the highly colored spots consisted mainly of fragments of seed coats. From this observation it was suspected that this formation of colored substances was due to an oxidizing enzyme, contained only in the seed coat, which was acting upon a chromogen, 3–4-dihydroxy-phenyl alanine, which occurs only in the cotyledons of the bean (**8**).

Several experiments were carried out in order to determine if this were the explanation of the color phenomena. Seed coats were carefully removed from several varieties of velvet beans so as to be entirely free from any interior portion of the bean. Also, cotyledons were carefully freed from any adhering seed coat. Each was then coarsely powdered.

It was found that none of the seed coats, alone, after long standing in water, produced any color. The same was true for the cotyledons of each

of the varieties examined. However, when the seed coat from any species or variety was mixed with the powdered cotyledons of that or of any other species or variety, in the presence of water, a pink color quickly appeared, usually within one minute, sometimes within half a minute or less, depending mainly upon the age of the beans and the degree of fineness of the powdered seed coats.

Action of the seed coats upon tyrosine

The seed coats from both the Early Speckled and Early White varieties produced, with a saturated aqueous solution of tyrosine, a series of colors similar to those formed when velvet bean cotyledons were used. The speed of the reaction, however, was much slower in the case of tyrosine.

Heating the dry seed coats at 105° C. for two hours diminished their activity but did not render them inactive. On the other hand, seed coats which had been mixed with water and heated in a boiling water-bath for fifteen minutes did not produce any color with velvet bean cotyledons in ten minutes. Usually, with fresh beans, color appears within half a minute.

Insolubility of the enzyme

In Water.—Seed coats were triturated with clean sand and water at ordinary temperature and filtered. The turbid filtrate when mixed with velvet bean interior showed very slight activity; the *clear* filtrate showed none. The residue, however, appeared to possess the usual degree of activity.

In Other Solvents.—Fifty per cent. alcohol, various mixtures of glycerine and water, dilute salt solutions, dilute alkali and dilute acids all failed to extract anything from the seed coats that showed enzyme activity. In all these cases the residue of seed coat proved to be active.

The influence of certain substances on the activity of the enzyme

A sample of coarsely powdered seed coat was allowed to stand four days in glycerine. After complete removal of the glycerine the seed coat was still very active in producing color with an aqueous extract of the velvet bean cotyledons; in the presence of fifty per cent. glycerine the activity of the enzyme was very much reduced. Color was produced in about seven minutes, while in the presence of water color appeared in one minute. This may have been due to the decrease in solubility of the chromogen and the slower rate of diffusion into the seed coat in the case of the glycerine.

Sodium chloride solutions decreased the speed of the reaction with increased concentration of the salt. With water, 1 per cent., 3 per cent., 5 per cent., 7 per cent. and 10 per cent. salt solutions, the time required to

produce noticeable change was, respectively, one minute, two minutes, two and a half minutes, four and a half minutes, ten minutes and a little more than ten minutes.

Potassium chloride produced, apparently, about the same effect as that produced by sodium chloride.

With 0.01 N mercuric chloride no color was produced in thirty minutes as against less than one minute for pure water.

With 0.01 N benzoic acid no color was observed in several hours.

With 0.2 N boric acid the speed of the reaction was reduced about fifty per cent.

Using tyrosinase from several different sources GORTNER (5) found that each of the phenols, orcin, resorcin, and phloroglucin, when added in small amount to the system, tyrosine + tyrosinase, completely prevented color formation. It has been found that these same phenols which carry two hydroxyl groups in the meta position to one another, also prevent the formation of color in a mixture of the velvet bean cotyledons and seed coats in the presence of water, or at least retard the action. In the absence of these phenols the appearance of a pink color has often been observed in less than a half minute.

INFLUENCE OF THE REACTION OF THE LIQUID ON THE ACTIVITY OF THE ENZYME

Using potato tyrosinase with tyrosine as substrate and working with buffered solutions, RAPER and WORMALL (9) found that the enzyme is most active between pH 6 and 8, and that activity ceases at pH 5 and 10.

With 3,4-dihydroxy-phenyl alanine probably no optimum pH is measurable for the velvet bean tyrosinase since very slight acidity prevents oxidation, while autooxidation occurs in even weak alkaline solutions.

Under conditions otherwise the same, 0.1 gm. of seed coat required five minutes to produce a given shade of pink color, while 0.5 gm. of seed coat required only one minute. The activity is, therefore, proportional to the mass of the seed coats used.

COMPARISON OF THE ACTIVITY OF BLACK (TRACY), MOTTLED AND WHITE SEED COATS

Using in each case the same quantity of seed coat and powdered cotyledons with a given volume of water it was found that the speed of action of the white seed coats was approximately twice as great as that of the mottled seed coats, and six to eight times that of the black seed coats. Seed coats from several samples of the black variety were used and in every case the formation of colored substances took place much more slowly than with mottled or white seed coats.

When white seed coats are mixed *in vitro* with powdered embryos of any variety of velvet bean, in the presence of water, they become uniformly dark colored.

Since the end product of the action of the oxidizing enzyme accumulates in the seed coat as the bean matures it might be expected that the more highly colored seed coats would show less activity than would be possessed by the mottled or white ones.

By cutting up mottled seed coats so as to separate the white portions from the highly colored portions it was found that the former brought about color formation more quickly than the latter. This shows that the enzyme is uniformly distributed throughout the seed coat, not in spots, and that the mottled effect is most probably due to uneven or irregular diffusion of the chromogen into the seed coat as the seed develops. In the white variety the chromogen does not seem to pass into the seed coat at all. In the black variety the chromogen evidently diffuses gradually and evenly into the seed coat, thus giving the uniform black color to the mature seed.

Influence of age on the activity of velvet bean seed coats

The cotyledons of new velvet beans are light colored, but they darken quite noticeably with age.

A sample of Early Speckled beans known to be several years old and having very dark interiors was powdered and mixed with water. Very little activity was shown; but by adding to the mixture some seed coat from a fresh sample of the same variety, considerable color formation took place in a few minutes, thus showing that it was the enzyme of the seed coat which had been affected by age.

A sample of Early White velvet beans known to be about six years old, when powdered and mixed with water, produced a pink color within five minutes. Usually, fresh beans of this variety, when so treated, show the pink color within one minute or less.

When the pink colored solution which is formed when powdered velvet beans are mixed with water is filtered from the solid material, the filtrate, on standing, passes through the same shades of color as those produced when the seed coats remain in the mixture, and also finally becomes black. Therefore, the presence of seed coats is not necessary for continuation of color formation.

The pink solution obtained by the action of MnO_2 or PbO_2 was also filtered and found to be oxidized through several shades of color, finally becoming black.

When powdered velvet beans are extracted with 95 per cent. alcohol and the solution allowed to stand in a cool place a solid separates which is almost

white. This gives the MOLISCH reaction for carbohydrate, but the carbohydrate may be removed by hot extraction with butyl alcohol, and the solid residue still gives all the reactions of 3,4-dihydroxy-phenyl alanine. When mixed with seed coats in the presence of water it shows the same color phenomena as is shown by an aqueous extract of the velvet bean cotyledons. It is evident that the chromogen extracted by means of alcohol produces the same color effects as do the aqueous extracts of the pulverized cotyledons.

Action of oxydizing enzymes from other sources

When petals of *Magnolia glauca* or *Magnolia grandiflora* are cut into strips and placed in a mixture of velvet bean cotyledons and water, a pink color is produced more quickly than by the velvet bean seed coats; and on standing, this mixture also passes through various shades of color, finally becoming black.

Aqueous solutions prepared from the petals produce the pink color even more quickly. Magnolia leaves also show a similar activity, but react more slowly. Also, sections of Irish potato or the filtered aqueous extract are about as active as the magnolia petals and produce practically the same color effects.

Agaricus campestris proved to be very active toward the chromogen of the velvet bean. Wheat bran, also, was quite active.

From the edible portion of the banana a solution of tyrosinase was prepared which almost instantly produced a pink color with an aqueous extract of velvet bean cotyledons. The peel of the banana was found to contain an insoluble tyrosinase which is active toward 3,4-dihydroxy-phenyl alanine.

A very active solution was also prepared from an undetermined species of mushroom having a white pileus with yellow gills.

Similar color changes are produced by some of the inorganic oxidizing agents. Thus with chemically pure manganese dioxide, or with lead dioxide, velvet bean cotyledons, in the presence of water, yield practically instantaneously a pink colored solution which passes through the usual series of colors, finally becoming black.

The pink colored solutions filtered from the residue of the dioxides, likewise, pass through the various shades of color ending in black.

It must not be supposed that the insoluble velvet bean tyrosinase is identical with the insoluble tyrosinase of the meal worm. While the enzyme of the velvet bean seed coat resembles that of the meal worm in its insolubility and in its action on tyrosine, it differs from it in having a slight oxidizing action on phloroglucinol, orcinol, pyrogallol and quinol, and by not losing its activity after standing for some time in glycerol.

Nitrogen and manganese content of the seed coats of the velvet bean

Nitrogen determinations of the seed coat of four varieties of velvet beans were made in the hope that they might throw some light on the chemical nature of the enzyme. If little or no nitrogen were present it would prove that this enzyme could not be protein-like. However, the minimum amount of nitrogen found was about 0.3 per cent. This is equivalent to about 1.87 per cent. protein, and if all the nitrogen were present in this form it would be quite possible for the enzyme to be a protein of some kind; on the other hand, it should be stated that the application of several protein tests failed to give satisfactory evidence of the presence of protein in the seed coats.

Although the nitrogen determinations did not throw any light on the chemical nature of the enzyme, yet they seem to have a bearing on the question of the deaminization of the chromogen of the velvet bean by the tyrosinase of the seed coat.

According to CHODAT and SCHWEIZER (**2**) tyrosinase deaminizes glycine, tyrosine and phenyl alanine. On the other hand, RAPER and WORMALL (**10**) did not obtain ammonia when tyrosinase acted upon tyrosine, and HAPPOLD and RAPER (**6**) were not able to detect the formation of ammonia when potato tyrosinase acted upon glycine, alanine or phenyl-amino-acetic acid, *unless* *p*-creosol or some other suitable phenol was present.

Judging from these results it is quite improbable that the tyrosinase of the velvet bean seed coat deaminizes 3,4-dihydroxy-phenyl alanine. In agreement with this we obtained no evidence of the formation of ammonia when velvet bean cotyledons and seed coats were mixed with water. One would naturally expect, then, that the seed coat of the black Tracy velvet bean would contain a higher percentage of nitrogen than is contained in the seed coats of the Early Speckled variety, since the latter contains much less melanin. For the same reason one would expect to find the nitrogen content of the Early White seed coats to be lower than that of the mottled varieties. On the contrary its nitrogen content is almost as high as that of the Osceola seed coat. This is not necessarily a contradiction. It was shown that the Early White seed coats showed decidedly greater enzyme activity than was shown by the seed coats of any other variety. This, of course, would mean that a larger amount of the enzyme is contained in this variety; and if the enzyme is a protein, as it may be, this would account for the higher nitrogen content of this seed coat.

The results shown in table I, based upon air dried material, were obtained by the Kjeldahl method for different samples of seed coats of the varieties indicated.

TABLE I

ANALYSES OF VELVET BEAN SEED COATS

VARIETY	NITROGEN	MOISTURE
	per cent.	*per cent.*
Tracy	0.662	10.09
	0.635	9.66
	0.624	
	0.626	
	0.608	
Osceola	0.343	11.58
	0.470	
	0.456	11.04
	0.463	
Early White	0.439	9.85
	0.426	
Early Speckled	0.301	10.78
	0.323	10.35
	0.335	11.14

Evidence was found that indicates the presence of more than one nitrogenous compound in the seed coat. A small quantity of seed coats of the Early Speckled variety was thoroughly extracted by each of the following solvents: water, 94 per cent. alcohol, and 0.2 per cent. aqueous solution of sodium hydroxide. The following amounts of nitrogen were extracted (Kjeldahl), based on air dried material: water, 0.014 per cent.; alcohol, 0.055 per cent.; 0.2 per cent. NaOH solution, 0.100 per cent. From these results it appears that almost all of the nitrogen of the seed coat exists in the form of an insoluble compound along with one or more other compounds present in small amount.

Analyses for manganese content of the seed coats were made because of the supposed relation of manganese to oxidase activity. In his investigations of laccase obtained from many different sources BERTRAND (1) showed that its activity was proportional to its manganese content. From *Medicago sativa* he obtained a preparation which was only slightly active and contained very little manganese. He found, however, that its activity was greatly increased by the addition of a small amount of manganese acetate or other manganese compound. From these results he concluded that manganese is an essential part of laccase.

EULER and BOLIN (3) later obtained a preparation of laccase from *Medicago sativa* which contained practically no manganese but was capable of oxidizing polyphenols in the presence of traces of manganese. Recently WIELAND and FISCHER (11) separated a plant oxidase from the juice of the fungus, *Lactarius vellereus*. Although they could detect no manganese in its ash, the enzyme was capable of oxidizing catechol and quinol, in the air, to the corresponding quinones.

From a consideration of these results and the fact that the velvet bean seed coat shows such pronounced enzymic activity, it was of interest to determine their manganese content. Table II shows amounts of manganese that were obtained by the periodate method, results being expressed in terms of the dry weight of the seed coats.

TABLE II

ANALYSES OF VELVET BEAN SEED COATS FOR MANGANESE

VARIETY	MANGANESE PER 100 GM. OF SEED COAT
	mg.
Early Speckled	23.10
Osceola	17.04
Tracy (black)	23.09
Chinese	16.15
Yokohama	16.15
Early White	23.31
Jack Bean	2.86

Although it can not be claimed that these results either confirm or disprove BERTRAND'S view, yet the finding of so much manganese in a tissue having pronounced enzyme activity may be considered very suggestive. It will be noticed that the black seed coat of the Tracy variety in which there is the greatest amount of pigment, and the seed coat of the white variety which proved to be most active, are the ones which contain the greatest amounts of manganese. These facts are at least in harmony with the view that manganese is concerned in the enzyme action.

The seed coat of the Jack Bean did not, as far as it was tested, show any enzyme action, and as is shown in the table, contains much less manganese than is contained in the velvet bean seed coats.

A comparison was made of the action of velvet bean seed coats and some other substances which act as oxidizing agents, such as manganese dioxide, lead dioxide, and benzoyl peroxide, on each of the following substances in the presence of water:

(1) Dihydroxy-phenyl alanine (aqueous solution from velvet bean cotyledons); (2) orcinol; (3) phloroglucinol; (4) pyrocatechol; (5) hydroquinone; (6) benzidine; (7) pyrogallol; (8) tincture of guaiac.

The results of these comparisons are summarized in the following paragraphs:

I. Dihydroxy-phenyl alanine.

(1) Velvet bean seed coat: pink color sometimes within twenty seconds, changing through several shades of red, brown, purple, finally becoming black.
(2) MnO_2: pink color practically instantaneously, quickly changing through various shades, finally black.
(3) PbO_2: pink color practically instantaneously, with changes similar to those in no. 1 and no. 2.
(4) Benzoyl peroxide: a purplish pink color observed after several hours.

II. Orcinol.

(1) Velvet bean seed coats: very slight pink after standing over night.
(2) MnO_2: slight red within one minute.
(3) PbO_2: slight color within two minutes. Reacted more quickly than the velvet bean seed coat, but more slowly than MnO_2.
(4) Benzoyl peroxide: very distinct pink in twelve hours.

III. Phloroglucinol.

(1) Velvet bean seed coat: very slight yellow after standing several hours.
(2) MnO_2: very distinct yellowish color after standing several hours.
(3) PbO_2: similar to no. 2.
(4) Benzoyl peroxide: faint yellow color within thirty minutes, but very little further change in twelve hours.

IV. Pyrocatechol.

(1) Velvet bean seed coat: yellowish color appeared very quickly. After several hours the seed coat was almost black but the solution remained slightly pink.
(2) MnO_2: greenish yellow color almost immediately, finally black.
(3) PbO_2: yellowish color very quickly. Solution brownish after several hours.
(4) Benzoyl peroxide: slight gray color within a few minutes; after standing over night, the liquid was yellowish brown.

V. Hydroquinone.

(1) Velvet bean seed coat: very slight pink color within twenty minutes, changing very slowly.
(2) MnO_2: deep reddish brown color within fifteen minutes.
(3) PbO_2: slight reddish color almost immediately, but changed very slowly.
(4) Benzoyl peroxide: very slight pink in five hours.

VI. Benzidine.

(1) Velvet bean seed coat: after standing twenty hours the liquid had not become colored, but the seed coats had taken on a slight purple color.
(2) MnO_2: slight pink very quickly.
(3) PbO_2: the liquid was colored pink very quickly.
(4) Benzoyl peroxide: bluish green color formed almost immediately. Became purple in a few minutes.

VII. Pyrogallol.

(1) Velvet bean seed coat: the solution was colored yellowish within two minutes; dark brown after twelve hours.
(2) MnO_2: brownish red solution almost immediately, black in twelve hours.
(3) PbO_2: after standing several hours there was somewhat more color than was produced by velvet bean seed coat.
(4) Benzoyl peroxide: no appreciable change within five minutes. Slight brown after long standing.

VIII. Tincture of guaiac.

(1) Velvet bean seed coat: some samples produced a very faint greenish color in a comparatively short time, others appeared to have no effect.
(2) MnO_2: a pale green produced quickly.
(3) PbO_2: result similar to that produced by MnO_2.
(4) Benzoyl peroxide: also produced a pale green very quickly.

On carefully examining the color reactions described here it is seen that the behavior of the velvet bean seed coat is in general similar in its effects to that of the inorganic dioxides and benzoyl peroxide. This similarity suggests the possibility that the enzyme of the velvet bean seed coat may itself be a peroxide.

Credit is due Mr. E. F. WILLIAMS for assistance in carrying out this investigation.

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LITERATURE CITED

1. BERTRAND, G. Sur l'action oxydante des sels manganeux et sur la constitution chimique des oxydases. Compt. Rend. Acad. Sci. Paris **124**: 1355–1358. 1897.
2. CHODAT, R., and SCHWEIZER, K. Über die deamidierende Wirkung der Tyrosinase. Biochem. Zeitschr. **57**: 430–436. 1913.
3. EULER, H., and BOLIN, I. Zur Kenntnis biologisch wichtiger Oxydationen. Zeitschr. physiol. Chem. **57**: 80–98. 1908.
4. GORTNER, R. A. A contribution to the study of the oxydases. Jour. Chem. Soc. **97**: 110–120. 1910.
5. ————. Studies on melanin III. The inhibitory action of certain phenolic substances upon tyrosinase. Jour. Chem. **10**: 113–122. 1911.
6. HAPPOLD, F. C., and RAPER, H. S. The supposed deaminising action of tyrosinase on amino acids. Biochem. Jour. **19**: 92–100. 1925.
7. LEHMANN, K. B., and SANO, Dr. Über das Vorkommen von Oxydationsfermenten bei Bakterien und höheren Pflanzen. Arch. Hyg. **67**: 99–113. 1908.
8. MILLER, EMERSON R. Dihydroxyphenylalanine, a constituent of the velvet bean. Jour. Biol. Chem. **44**: 481–486. 1920.
9. RAPER, H. S., and WORMALL, A. The tyrosinase-tyrosine reaction. Biochem. Jour. **17**: 454–469. 1923. (See also Physiol. Rev. **8**: 256. 1928.)
10. ————. The theory of deamination. Biochem. Jour. **19**: 84–91. 1925.
11. WIELAND, H., and FISCHER, F. G. Zur Frage der katalytischen Dehydrierung. Ber. deutsch. chem. Ges. **59**: 1180–1191. 1926.

THE DETERMINATION OF NITRATE IN GREEN TOMATO AND LETTUCE TISSUES

E. M. EMMERT

A method for the rapid and accurate determination of nitrate nitrogen in fresh plant tissues was desired in connection with some experiments on the absorption of nitrate by tomato and lettuce plants, but none seemed available. The reduction methods with Devarda alloy have been shown by RANKER (10) and others to be inaccurate. Other reduction methods seem to be subject to the same inaccuracies or are very laborious. The method in which nitric acid is reduced to nitric oxide by ferrous chloride, and the nitric oxide caught over alkali and absorbed by permanganate (9), may be accurate for fairly large amounts of nitrate, but is too laborious and requires large samples. The method suggested by EMMERT (2) in which the nitric acid is distilled from 50 per cent. by volume sulphuric acid and caught in an oxidizing solution of chlorine dioxide to reoxidize lower oxides of nitrogen, is rather laborious.

The phenoldisulphonic method seems to be the most promising method for small amounts of nitrates in soils (5). This method has been applied to plants by BURRELL and PHILLIPS (1). An alcohol extract was used, making it necessary to clear with lead acetate and destroy organic matter with sodium peroxide. Their results are very favorable, but still the process is laborious. Trials were made on water extracts by the writer. It was soon found that some agent for clarifying and stopping reduction was needed. Most gratifying results were secured by triturating green tissues with calcium hydroxide and filtering from copper hydroxide. By this means a surprisingly clear aqueous extract was secured which gave little or no interfering color with phenoldisulphonic acid. Data are presented which show that enzymes, sugars, and other reducing substances in the amounts found in tomato tissues do not reduce nitrate in solutions made strongly alkaline with calcium hydroxide.

The use of charcoal as advocated by GILBERT (4) for clearing the solution, does not seem advisable, since reduction is not stopped and extreme care has to be taken to obtain the proper form of charcoal, for occlusion and absorption cause errors, as indicated by HARPER (5).

During the writing of the present paper, a method appeared in a recent issue of PLANT PHYSIOLOGY, by HOLTZ and LARSON (6), which is somewhat similar to the method of this paper. Their method was used on dry tissue, however, and included the use of charcoal, thus subjecting itself to the dangers of GILBERT'S method. Their leaching process is much longer than trituration and it does not seem as thorough in extracting nitrate as does

trituration of fresh tissue. In any drying process, changes occur no matter how carefully it is carried out. A short method of determining nitrate in green tissue enables the almost immediate analysis of tissue which has not had a chance to change to any extent. HOLTZ and LARSON may have secured complete recovery of nitrate added, yet this does not prove that all their tissue may not have suffered approximately equivalent loss of nitrate in the process of drying and during the analysis. Work with nitrate-free plant material should overcome such constant factor occurring undetected during the analysis.

It is not claimed that the method presented in this paper will give clear extracts on tissues other than those used, but if it clears these tissue extracts satisfactorily it is safe to suppose that it will be of value in other tissues.

Plants grown in normal soils do not usually contain enough chloride to interfere, so that the removal of chloride may be omitted in most cases.

Procedure

The sample is detached from the growing plant and placed in a covered container. One gram (larger amounts can be used if reagents are increased proportionately) is weighed out at once. Within fifteen minutes after weighing, the one gram sample should be placed in a mortar, 0.5 gram calcium hydroxide added, and the mixture triturated until a green (if chlorophyll is present) paste results. Care should be taken to break up all particles of tissue. Place 5 cc. of M/2 molar copper sulphate in a 50-cc. graduate and make up to exactly 50 cc. Add this to the paste in the mortar and mix thoroughly. After 5 minutes filter off a 25-cc. aliquot (a larger aliquot should be used if nitrate is low), discarding the first few cc. of filtrate. Evaporate to dryness without overheating the residue and determine nitrate by the phenoldisulphonic acid method, using sodium hydroxide as the base. It will be necessary to filter the yellow solution for reading. Addition of calcium hydroxide before filtering gives a better solution, since calcium sulphate is only slightly soluble and a precipitate of sodium sulphate does not form on standing. If more than 20 ppm. of chloride is present it must be removed by treatment with silver sulphate, as outlined by HARPER (5). Further protection against reduction may be secured by adding a cubic centimeter of toluene to the tissue at the time of trituration.

Principles involved

On trituration with calcium hydroxide the plant cells are broken up and all acidity is neutralized, free nitric acid, if present, being converted to $Ca(NO_3)_2$ before it can be reduced, which would likely take place if the

sample were triturated alone. Enzyme action probably is stimulated by trituration and, unless calcium hydroxide is present, rapid reduction of nitrate occurs (3) (7). The highly absorptive properties of copper hydroxide enable it to absorb practically all interfering substances leaving a clear solution of nitrate with traces of sugars, minerals and other substances of the plant sap. These amounts are so small that the phenoldisulphonic acid method for nitrates was found to give accurate results.

TABLE I

NITRATE NITROGEN FOUND IN UPPER LEAVES OF TOMATO BY TRITURATION WITH CALCIUM HYDROXIDE—PERCENTAGE OF GREEN WEIGHT

PLOT	DET. 1	DET. 2	DIFFERENCE
	per cent.	*per cent.*	*per cent.*
1	0.040	.....	.....
2	0.0130	0.0142	0.0012
3	0.0166	0.0183	0.0017
4	0.0107	0.0142	0.0035
5	0.0081	0.0069	0.0012
6	0.0094	0.0088	0.0006
7	0.0108	0.0100	0.0008
8	0.0075	0.0075	0.0000
9	0.0085	0.0081	0.0004
10	0.0380	0.0400	0.0020
11	0.0081	0.0085	0.0004
12	0.0262	0.0238	0.0024
13	0.0250	0.0212	0.0038
14	0.0338	0.0338	0.0000
15	0.0262	0.0288	0.0026
16	0.0238	0.0175	0.0063
17	0.0276	0.0250	0.0026
18	0.0138	0.0100	0.0038
19	0.0300	.....	.....
20	0.0108	0.0117	0.0009
21	0.0100	0.0133	0.0033
22	0.0092	0.0083	0.0009
23	0.0188	0.0188	0.0000
24	0.0375	0.0212	0.0163
25	0.0138	0.0175	0.0037
26	0.0092	0.0125	0.0033
27	0.0108	0.0125	0.0017
28	0.0166	.....	.....
29	0.0133	0.0117	0.0016
30	0.0188	0.0163	0.0025
31	0.0150	0.0125	0.0025
32	0.0092	0.0092	0.0000
33	0.0200	0.0117	0.0083

Results

In tables I–III are presented series of nitrate determinations on tomato leaves, (I); tomato stems, (II); and lettuce leaves, (III). The soil in the plots was treated to control the reaction. It was found impossible to obtain uniform samples as to age and percentage of veins. Samples of tissue taken from the same plot might be several days or several weeks different in age, depending on their positions. Upper leaves gave 0.025 per cent. nitrogen, while lower leaves of the same plant contained 0.110 per cent. Samples taken from the same leaf might differ, since the veins contain a much larger per cent. of nitrate nitrogen and it is hard to secure the same proportion of veins in each case.

TABLE II

NITRATE NITROGEN FOUND IN TOMATO STEMS BY TRITURATION WITH CALCIUM HYDROXIDE—PERCENTAGE OF GREEN WEIGHT

PLOT	DET. 1	DET. 2	DIFFERENCE
	per cent.	*per cent.*	*per cent.*
7	0.095	0.095	0.0
8	0.100	0.100	0.0
9	0.120	0.120	0.0
10	0.110	0.110	0.0
11	0.110	0.110	0.0
12	0.140	0.140	0.0
14	0.140	0.145	0.005
18	0.115	0.110	0.005
20	0.125	0.115	0.010
22	0.105	0.100	0.005
23	0.115	0.115	0.0
24	0.115	0.115	0.0
25	0.120	0.120	0.0
26	0.115	0.115	0.0
27	0.130	0.135	0.005
28	0.140	0.135	0.005
29	0.140	0.140	0.0
30	0.145	0.145	0.0
32	0.120	0.120	0.0

The difference between leaf and vein tissue in lettuce is shown by the fact that a sample of veins gave 0.115 per cent. nitrate nitrogen, while one from the margin of the leaves gave 0.055 per cent. Considering these points it is not justifiable to attribute the variations in tables I and III to the method. It seems more probable that they are due to differences in the samples. The fact that similar treatments were consistent in showing

TABLE III

NITRATE NITROGEN FOUND IN LETTUCE LEAVES BY TRITURATION WITH CALCIUM HYDROXIDE—PERCENTAGE OF GREEN WEIGHT

PLOT	DET. 1	DET. 2	DIFFERENCE
	per cent.	*per cent.*	*per cent.*
1	0.250	0.237	0.013
2	0.115	0.110	0.005
3	0.085	0.080	0.005
4	0.075	0.080	0.005
5	0.090	0.100	0.010
6	0.110	0.110	0.0
7	0.113	0.113	0.0
8	0.131	0.134	0.003
9	0.125	0.131	0.006
10	0.113	0.119	0.006
11	0.125	0.100	0.025
12	0.093	0.081	0.012
13	0.100	0.115	0.015
14	0.119	0.125	0.006
15	0.113	0.119	0.006
16	0.119	0.125	0.006
17	0.100	0.113	0.013
18	0.125	0.125	0.0
19	0.125	0.134	0.009
20	0.188	.....	.....

like nitrate nitrogen content in most of the cases shows that the method must have a greater degree of accuracy than the data exhibit.

In table II the stems of suckers were used. In this case it was easier to secure uniform samples by using thin cross slices of stems of about the same diameter and apparent age (stems of about 0.5 cm. in diameter were used for the determinations reported in table II). The samples were handled rapidly to avoid moisture losses. Here the variation is below 5 per cent. in every case but one. This table shows that the method gives consistent results with stem tissue. The question of water loss is the big factor in variation here. As soon as the slices are cut they must be weighed at once, preferably in a covered container.

Recovery of nitrate

In order to further check the method, one gram samples of tomato leaf tissue were weighed and 5 cc. of standard nitrate solution were added to the tissue along with the calcium hydroxide. The sample was triturated and 40 cc. of water, plus 5 cc. M/2 $CuSO_4$ solution added. A sample of the same

TABLE IV

RECOVERY OF ADDED NITRATE NITROGEN BY TRITURATION WITH CALCIUM HYDROXIDE
ONE GRAM OF GREEN LEAVES OF TOMATO PLANTS + 0.5 MG. NITRATE NITROGEN ADDED AS KNO_3

PLOT	N	N	N RECOVERED	DEVIATION* FROM 0.5 MG.
	mg.	*per cent.*	*mg.*	*mg.*
1	0.9	0.090	.....	.....
1 + N	1.43	.....	0.53	+ 0.03
2	0.85	0.085	.....	.....
2 + N	1.30	.....	0.45	− 0.05
3	0.60	0.06	.....	.....
3 + N	1.10	.....	0.50	0.00
4	0.9	0.090	.....	.....
4 + N	1.4	.....	0.50	0.00
5	0.58	0.058	.....	.....
5 + N	1.10	.....	0.52	+ 0.02
6	0.60	0.06	.....	.....
6 + N	1.10	.....	0.50	0.00
7	0.50	0.05	.....	.....
7 + N	1.07	.....	0.57	+ 0.07
8	0.38	0.038	.....	.....
8 + N	0.85	.....	0.47	− 0.03
9	0.55	0.055	.....	.....
9 + N	1.05	.....	0.50	0.00
10	0.38	0.038	.....	.....
10 + N	0.90	.....	0.52	+ 0.02

* Total + deviation = 0.14 in four cases.
Total − deviation = 0.08 in two cases.
Average deviation = 0.037.
Per cent. of error = 7.4.

TABLE V

EFFECT OF REDUCING SUGARS AND SUCROSE ON RECOVERY OF NITROGEN ADDED AS POTASSIUM NITRATE

SAMPLE	NITROGEN ADDED	NITROGEN FOUND
	mg.	*mg.*
Standard solution alone	0.5	0.5
Standard solution + 3 mg. sucrose	0.5	0.5
Standard solution + 3 mg. glucose and levulose	0.5	0.5
Standard solution + 6 mg. sucrose	0.5	0.5

leaf was then analyzed in the usual way. Table IV shows the recoveries made. In four out of ten cases exact recovery was made. In only two cases was the deviation as much as 10 per cent. of the added nitrogen. In two cases the deviation was 6 per cent., and in the other two, 4 per cent. However, in only two cases was there actual loss, which would lead one to believe that the fluctuation must have been due to something besides loss of nitrate nitrogen in the procedure. The recovery of nitrate added to nitrate-free plant tissue was equally good (see table VI), the variation ranging from −10 to +16 per cent. of the added nitrate in 12 experiments, with four cases of exact recovery.

Interfering colors

In the determinations on plants containing normal amounts of nitrate no interfering colors became evident, the unknown matching the standard in excellent manner. If, however, the residue from the evaporation was overheated a faint caramel color developed, showing the presence of small amounts of organic substances. Evidently the amount of organic matter was much less than that found by BURRELL and PHILLIPS (**1**) using an alcohol extract. It is natural to suppose that alcoholic extracts would contain more organic matter. Alcohol also dissolves chlorophyll which is a strong reducing material, and it would naturally promote reduction of nitrate. Water, on the other hand, would not dissolve chlorophyll nor other organic substances to the degree that alcohol does. At any rate, interfering colors did not seem to be a source of error.

In the experiments reported in table VI, however, when only minute traces of nitrate were present, if any, some instances of interfering color occur, but in most cases the color was not enough to be significant. In table V interference by an amount of carbohydrate found in normal tomato tissue was not detected. A sugar solution containing 3 mg. of sugar (shown later to be the amount present in one gram of normal tomato leaves) was run through as a blank to note if any color developed when it was certain no nitrate was present. The solution after adding NaOH was perfectly colorless. The same thing was true of 6 mg., but 12 mg. of sugar gave a slight coloration, which was of a decidedly different yellow from that of the standard.

One point should be noted here, however. When ammonium hydroxide was used as the base for neutralizing the phenoldisulphonic acid, a slight color interference appeared. When sodium hydroxide was used, and the solution filtered, a solution admirably adapted for colorimetric readings was secured. On standing, a further precipitate may settle out. If calcium hydroxide is added before filtration and shaken well, calcium sulphate is

formed. Since calcium sulphate has about the same solubility, regardless of temperature, the solution remained clear on standing.

Reduction of nitrate

In strongly alkaline solution there seems to be no reduction by reducing materials present in plants. Table V indicates no loss from sugars added to nitrate solutions. A 5 per cent. solution of fresh sodium sulphate was added to a sample of standard containing 0.5 gram calcium hydroxide and the solution evaporated. Very little, if any, reduction occurred. However, when sodium hydroxide and metallic zinc were used in the presence of calcium hydroxide, the reduction was more than half.

In table V the amounts of sucrose and hydrolyzed sucrose to be added were determined by adding together the figures found by KRAUS and KRAYBILL (**8**) for sucrose and reducing substances in tomato leaves. Their series M was chosen, since it was the nearest to the type of soil used for the tomatoes analyzed by the writer. Since a one gram sample of tomato tissue was used for the trituration method and the highest amount of reducing substances and sucrose found by KRAUS and KRAYBILL was 0.3 per cent. of the green weight, a total of 3 mg. of sucrose were added. Reducing sugars were introduced by hydrolyzing with dilute acid. Some hydrolysis likely took place on heating the alkaline solutions of sugar and nitrate.

Table V shows that sugars had no reducing action on nitrate in the presence of calcium hydroxide. Of course, this does not prove that there are not enzymes or other substances in plants which might reduce the nitrate.

ECKERSON (**3**) finds that reduction in plant tissues takes place at pH 8.4 to pH 9.2, though pH 7.6 is optimum. Her figures seem to show that strong acidity checks the reducing action almost better than strong alkalinity. KASTLE and ELVOVE (**7**), on the other hand, found that reduction was prevented by lime-water or NaOH. Of course the amount of calcium hydroxide used in the present method would create a pH well above 10 and the excess present would maintain it thus.

In view of the contradictory findings on this point in the literature it was decided to test reduction by plant tissue still further. Although table IV gave almost complete recovery of nitrate nitrogen added, it was thought that a constant source of reduction might make it appear like complete recovery, when in reality a certain constant amount was lost in all determinations. If, however, known amounts of nitrate nitrogen were added to nitrate free tissue and complete recovery was secured, no constant error could be present. Consequently, garden bean seeds were germinated in pure white sand free of nitrate. When the cotyledon leaves were at their maximum the tissue was used. There would be no chance for nitrate nitrogen here,

TABLE VI

RECOVERY OF NITRATE ADDED TO NITRATE-FREE PLANT TISSUES

SAMPLE	N AS NITRATE ADDED	N AS NITRATE RECOVERED	NOTES ON COLOR
	mg.	*mg.*	
Bean	None	Faint trace of color	Seems to be faint nitrate color
"	None	Faint trace of color	Just a trace of cloudiness
"	0.1	0.100	Slight interference but reading was definite
"	0.1	0.100	No interference
"	0.2	0.188	No interference
"	0.2	0.180	Slight interference
"	0.5	0.500	No interference
"	0.5	0.500	No interference
"	1.0	0.950	No interference
"	1.0	1.050	No interference
Tomato	None	Trace of color	More color than in bean, but not significant
"	1.0	0.161	Slight interference
"	0.2	0.206	No interference
"	0.5	0.500	No interference
"	1.0	0.950	No interference

since the roots could not absorb any and it is pretty certain that all nitrogen in seeds is in the reduced form. Nitrate-free tomato tissue was secured by washing out the roots of about 8-inch plants and placing them in pure white sand. After a week considerable nitrate was still evident, as shown by the method used in the present paper, but in two weeks' time the nitrate present was negligible.

The recoveries given in table VI are rather favorable, although in some cases a little nitrogen seems to be lost. However, this is almost balanced by the cases in which too much nitrogen was found. Considering the fluctuation possible in making colorimetric readings, the writer feels that little reduction takes place in the presence of calcium hydroxide in the procedure for nitrate suggested.

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LITERATURE CITED

1. BURRELL, R. C., and PHILLIPS, T. G. The determination of nitrate nitrogen in plants. Jour. Biol. Chem. **65**: 229–234. 1925.

2. EMMERT, E. M. The chlorate method for the determination of nitrate nitrogen, total nitrogen, and other elements in soils and plant tissues. Jour. Assoc. Offic. Agr. Chem. **12**: 240–247. 1929.
3. ECKERSON, S. H. Protein synthesis by plants I. Nitrate reduction. Bot. Gaz. **77**: 377–390. 1924.
4. GILBERT, B. E. The adaptation of certain colorimetric methods to the estimation of nitrates, phosphates, and potassium in plant solutions. Plant Physiol. **1**: 191–199. 1926.
5. HARPER, H. J. The accurate determination of nitrates in soils. Jour. Ind. Eng. Chem. **16**: 180–183. 1924.
6. HOLTZ, H. F., and LARSON, CARL. A simple method for nitrate nitrogen determination in wheat plants. Plant Physiol. **4**: 288–291. 1929.
7. KASTLE, J. H., and ELVOVE, E. On the reduction of nitrates by certain plant extracts and metals, and the accelerating effect of certain substances on the progress of the reduction. Amer. Chem. Jour. **31**: 606–641. 1904.
8. KRAUS, E. J., and KRAYBILL, H. R. Vegetation and reproduction with special reference to the tomato. Oregon Agr. Exp. Sta. Bull. 149. 1918.
9. MITCHELL, H. H., SHONLE, H. A., and GRINDLEY, H. S. The origin of the nitrates in the urine. Jour. Biol. Chem. **24**: 461–490. 1916.
10. RANKER, E. R. Some inaccuracies of the Devarda method when applied to plant materials. Jour. Assoc. Offic. Agr. Chem. **10**: 252–256. 1927.

MOISTURE FLUCTUATIONS IN EXTRACTED PLANT SOLUTIONS AND IN LEAF TISSUE[1]

BASIL E. GILBERT AND WALDO L. ADAMS

For a number of years workers at the Rhode Island Agricultural Experiment Station have made chemical and agronomical studies of the quantities of nutrient mineral elements necessary to insure optimum growth of field and market garden crops. Recently attempts have been made to measure the amounts of these elements found in the plant (**3, 4**) at various stages of growth as related to varying environmental conditions such as fertilization and weather factors. This localization of attack has involved the use of certain colorimetric chemical methods to measure small concentrations of nutrient elements found in the plant solution. Concentration has been expressed as parts per million of plant solution. Since an aqueous solution is being considered, the question arose as to the influence of environmental factors such as evaporation and soil moisture upon the concentration of the plant solution. Might there not be sufficient fluctuations in total moisture to introduce appreciable errors in the concentrations of nutrient elements in the plant solution? In order to answer this question it seemed necessary to make studies of the fluctuations occurring in the moisture content of the crop plant.

There seemed to be two fairly clear-cut ways of attacking this problem: (1) To determine the water content of the extracted plant solution at various times during the growing period. (2) To determine the total moisture content of crop plants over an entire season of growth.

Moisture content of 1926 plant solutions

In order to determine the water content of the extracted plant solution the refractometric method suggested by GORTNER and HOFFMAN (**5**) was used. By this method the moisture content of expressed plant tissue fluids is determined by difference, the Abbé refractometer measuring the percentage of total solids in the expressed sap in units of the sugar scale. This method is based on the fact that the refractive indices of solutions of inorganic salts and proteins in the concentrations normally present in plant sap are very nearly the same as the indices of similar solutions of carbohydrates. One or two drops of the solution expressed in the manner described by GILBERT (**2**) were used for each determination and the determinations were made in duplicate.

[1] Contribution no. 376 of the Rhode Island Agricultural Experiment Station.

By means of this method the average moisture percentages for the crops and tissues given in table I were determined. The crops listed were all grown on Merrimac silt loam on the Rhode Island experimental plats and in the humid climate of the coastal region.

From table I it will be noted that the standard deviation which measures the absolute variation from the mean is very small in each case. This is of interest since the determinations were made throughout the length of a growing season during which varying conditions of evaporation and soil moisture were obtained. A chart giving a description of weather observations at the Rhode Island experimental plats for the season of 1926 has already been published (**4**).

TABLE I

AVERAGE MOISTURE PERCENTAGES FOUND IN SOLUTIONS OF CROPS GROWN UNDER FIELD CONDITIONS IN 1926

CROP	TISSUE	NUMBER OF DETERMINATIONS	AVERAGE MOISTURE	STANDARD DEVIATION
			per cent.	
Parsnip	Roots	11	89.7 ± 0.4	2.0 ± 0.3
Beet	Roots	24	92.8 ± 0.2	1.4 ± 0.1
Carrot	Roots	19	93.5 ± 0.1	0.9 ± 0.1
Rutabaga	Roots	21	95.4 ± 0.2	1.3 ± 0.1
Celery	Stalks and leaves	38	95.1 ± 0.2	1.4 ± 0.1
Beet	Stalks and leaves	26	97.0 ± 0.1	0.7 ± 0.1
Buckwheat	Stalks and leaves	18	97.9 ± 0.1	0.6 ± 0.1
Spinach	Entire leaves	12	97.9 ± 0.2	1.1 ± 0.2
Lettuce	Entire leaves	21	97.5 ± 0.2	1.1 ± 0.1
Tomato	Branch tips	12	92.8 ± 0.3	1.4 ± 0.2
Potato	Branch tips	8	94.0 ± 0.4	1.7 ± 0.3
Corn	Tissue of stalk above uppermost node	11	96.4 ± 0.4	2.0 ± 0.3
Cabbage	Leaves minus midribs	47	95.1 ± 0.1	1.3 ± 0.1

The determinations were also made upon tissues of crops growing with both optimum and suboptimum commercial fertilization and on manured and non-manured situations. Environmental conditions, therefore, seem to have had little effect upon the concentration of the expressed plant solution. The lack of significance is also seen if the deviations are considered in the determination of an element which is present in the solution in magnitudes of 100 parts per million of solution as is the case with the nitrate ion (**3**).

Determinations of total moisture in 1927 and 1928

Methods

In order to determine the total moisture, the Bidwell-Sterling direct distillation method (1) was employed. This consists of weighing the tissue immediately after separating it from the plant, plunging it into moisture-free toluene, distilling, collecting the resulting water and reading the volume of water.

From the original weight and the volume of distilled water, the percentage of total moisture can be calculated. For plant tissue, since an appreciable error can easily be caused by exposure of cut surfaces to the air, tared weighing bottles were used. An estimated amount of tissue was introduced into the weighing bottles and tightly stoppered with a ground glass cover to prevent loss of moisture. The bottles were brought to the laboratory and weighed, and bottles and tissue were immersed in toluene. A further source of error with this method is the loss of vapor through faulty corks during distillation. This was overcome by the use of a composition cork manufactured by the Armstrong Cork Company.

In order to gain an idea as to the accuracy of this method when applied to plant tissue, several determinations were made on the same day with beet leaf blades grown in the greenhouse.

It will be seen from table II that the error of the method is small, the greatest spread being 1.5 units from the average of six determinations.

TABLE II

Bidwell-Sterling moisture determinations. Leaf blade tissue of beets grown in the greenhouse

Determinations	Total moisture
	per cent.
Ia	89.6
b	90.8
IIa	90.8
b	90.2
IIIa	88.6
b	88.1
Average total moisture,	89.6

Moisture content of different tissues

In an endeavor to minimize sampling errors, comparative determinations were made on beet leaf blades and petioles; on beet leaf midribs; and on the remaining portions of the leaves after the midribs were removed.

In table III the total moisture contents as found in blades and petioles are compared. In order to evaluate the differences found, STUDENT'S method (**6**) has been used. By this method odds of 30:1 are usually considered significant. It will be noted that a significant difference was found when the midrib was removed from the leaf tissue.

TABLE III

PERCENTAGES OF TOTAL MOISTURE IN BEET TISSUE GROWN IN THE GREENHOUSE

LEAF BLADES	LEAF PETIOLES	MIDRIB	REMAINING PORTION
87.3	86.2	89.8	92.3
88.4	89.8	90.6	92.0
88.6	88.6	86.5	86.1
87.6	88.9	89.0	90.7
89.5	92.1	89.7	95.4
89.9	93.1	86.6	87.3
		85.8	89.4
		85.3	93.9
Average ... 88.5	89.8	87.9	90.9
Odds 16:1		Odds 79:1	

Further proof of the importance of this procedure is given by the lack of significance of odds of 16:1 when leaf blades, containing midribs were compared with petioles. An average difference of 3 per cent. total moisture was found between midribs and the remaining portions of the blades. Thus, to avoid greater sampling error and considering the higher moisture content of the midrib, it has been considered wise to remove it in the case of crops where the midrib tissue is large in quantity. By this means also a tissue with less differentiation is secured and one which is more actively concerned with the processes of metabolism. This latter point is of value as in former chemical determinations upon the plant solution, an attempt has been made to select tissues which might be considered as localized areas of active metabolism.

MAXIMUM MOISTURE CHANGES

In order to assemble data concerning the loss of moisture as the plant tissue approaches the wilting condition, barley and Swiss chard were grown in sand cultures in greenhouse benches. When both crops had grown to an average height of approximately 8 inches and good leafy growth was secured, the areas were divided into equal parts. For each crop, water was supplied to one area daily while the other area was not watered. Daily de-

terminations of moisture in the leaves of barley and in the half leaves (midribs removed) of chard were made. All weighings were made immediately after severing the leaves from the plants.

In table IV the total moisture determinations on both crops are given. It will be noted that when serious wilting was observed the greatest percentage difference between the watered and unwatered plants was 7.3 per cent. with barley and 7.6 per cent. with Swiss chard. In neither case had

TABLE IV

PERCENTAGE OF TOTAL MOISTURE IN LEAF TISSUE OF CROPS GROWN IN THE GREENHOUSE

DATE	CROP	TISSUE	WATERED	UNWATERED	DIFFERENCE
Dec. 30	Barley	Entire leaf	87.4	87.3	+0.1
Jan. 11	Barley	Entire leaf	84.9	83.6	+1.3
Jan. 12	Barley	Entire leaf	86.4	83.6	+2.8
Jan. 13	Barley	Entire leaf	85.8	81.1	+4.7
Jan. 14	Barley	Entire leaf	84.1	78.6*	+5.5
Jan. 18	Barley	Entire leaf	83.6	82.9	+0.7
Jan. 20	Barley	Entire leaf	84.8	77.5*	+7.3
Jan. 16	Swiss chard	Leaves minus midrib	82.4	82.8	−0.4
Jan. 17	Swiss chard	Leaves minus midrib	89.2	88.9	+0.3
Jan. 19	Swiss chard	Leaves minus midrib	85.6	81.9	+3.7
Jan. 31	Swiss chard	Leaves minus midrib	93.9	90.0	+3.9
Feb. 1	Swiss chard	Leaves minus midrib	91.6	87.2	+4.4
Feb. 2	Swiss chard	Leaves minus midrib	89.8	86.4	+3.4
Feb. 3	Swiss chard	Leaves minus midrib	90.8	83.2*	+7.6

* Plants seriously wilted.

wilting conditions become severe enough to prevent recovery of turgor when water was supplied. Such a degree of wilting, however, is seldom seen under mesophytic field conditions such as are usual at the Rhode Island experiment station.

TOTAL MOISTURE FLUCTUATIONS IN 1928 FIELD CROPS

In order to study total moisture fluctuations under field conditions, determinations were made during the growing season of 1928 with leafy tissue of beet, tomato, celery, and corn.

With beet and corn the sampling was done so that the midrib was omitted. In sampling each of these crops, a portion of one leaf only, or a leaflet, from an individual plant was taken. The number of plants to be

sampled was determined by the relative weight of the leafy tissue and varied as follows:

	Number of plants
Tomato—leaflets near branch tips	25
Celery—leaflets near branch tips	25
Corn—cross-sections near center of lateral half of leaf-blade	50
Beet—cross-sections near center of lateral half of leaf-blade	30

These crops were grown under the same nutrient environmental variations as have already been described for the 1926 crops. Records of rainfall, soil moisture, and evaporation (by means of standard atmometers) were kept. The only correlation found was between total moisture in tissue and evaporation, there being a definite depression in the total moisture of tomato and corn leaves from July 25 to August 10, which was accompanied by conditions of quite high evaporation. However, at no time during this period was wilting observed in these crops, and the total depression in the moisture curve was no greater than 7 per cent.

In so far as was possible all tissue sampled was kept comparable with regard to size of leaf chosen, position on plants, and portion of leaf tissue. The effect of progressive maturity upon moisture content became very evident with corn. Toward the latter part of the season the percentage of moisture became materially less and as a result the standard deviation for the entire season is higher than with beet and celery (table V). This is to be expected as a very definite drying of the leaf tissue was observed visually as the season progressed.

Referring to table V it will be seen that with the four crops used in this study the standard deviations from the mean total moisture over the periods studied are very small. From this fact the conclusion may be drawn that

TABLE V

AVERAGE TOTAL MOISTURE IN CROPS GROWN UNDER FIELD CONDITIONS IN 1928

CROP	SAMPLING PERIOD	TISSUE	NUMBER OF DETERMINATIONS	TOTAL MOISTURE	STANDARD DEVIATION
	weeks			*per cent.*	
Beet	Sixth to twelfth	Leaves minus midribs	18	86.8 ± 0.4	2.7 ± 0.3
Tomato	Sixth to fifteenth	Leaflets near branch tips	32	80.8 ± 0.5	4.1 ± 0.3
Celery	Sixth to eleventh	Leaves minus midribs	32	78.5 ± 0.3	2.7 ± 0.2
Corn	Eighth to thirteenth	Leaves minus midribs	23	73.5 ± 0.7	4.7 ± 0.5

any error brought about in the chemical estimation of the concentration of nutrient elements in the plant solution might also be expected to be small. On a basis of 300 parts per million of nitrate ion, which is a desirable amount to maintain normal growth as shown in a former paper (3), and a maximum total moisture deviation of 5 per cent., an error of 15 parts per million would be introduced. Until the methods used in connection with studies of plant solutions are refined greatly this source of error may be disregarded when considered in connection with crops grown in normal mesophytic conditions.

Summary

Associated with studies of the quantities of certain mineral nutrient elements found in plant tissue solutions under varying conditions of nutrition and environment, data have been secured on the fluctuations of moisture in the same tissue.

1. By means of the refractometer, seasonal fluctuations in the percentage of water in the extracted tissue solution were studied.

2. The total moisture content of beet-leaf tissue has been studied and comparisons made between the content of blades, midribs, and petioles. The difference between the average moisture content of petioles and blades was found to be insignificant while midribs contained 3.0 per cent. more moisture than the remaining portion of the blade.

3. A study of the total daily moisture fluctuations in the leaf tissue of Swiss chard and barley grown under glass were made. By withholding water from a portion of each crop maximum differences of 7.6 and 7.3 per cent., respectively, in moisture content was found with both crops when wilting conditions were obtained.

4. Comparisons of soil moisture, evaporation from atmometers, and total moisture in leaf tissue were made with crops growing under field conditions. Evaporation, alone, showed evidence of correlation with moisture depression.

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LITERATURE CITED

1. Bidwell, G. L., and Sterling, W. F. Preliminary notes on the direct determination of moisture. Jour. Assoc. Off. Agr. Chem. **8**: 295–301. 1924–1925.
2. Gilbert, B. E. The adaptation of certain colorimetric methods to the estimation of nitrates, phosphates and potassium in plant solutions. Plant Physiol. **1**: 191–199. 1926.

3. ————————, and HARDIN, LEO J. The current mineral nutrient content of the plant solution as a possible means of chemical control of optimum fertilization. Jour. Agr. Res. **35**: 185–192. 1927.
4. ————————, MCLEAN, F. T. and ADAMS, W. L. The current mineral nutrient content of the plant solution as an index of metabolic limiting conditions. Plant Physiol. **2**: 139–151. 1927.
5. GORTNER, R. A., and HOFFMAN, W. F. Determination of moisture content of expressed plant tissue fluids. Bot. Gaz. **74**: 308–313. 1922.
6. "Student." The probable error of a mean. Biometrika **6**: 1–25. 1908.

THE EFFECT OF pH VALUE ON THE INACTIVATION TEMPERATURE OF FRUIT OXIDASE

W. Y. Fong and W. V. Cruess

The inactivation temperature of the oxidase of fruits is of interest in connection with the processing of fruits and fruit products by heat, as inactivation of the oxidase is essential to retention of the original fruit color.

Overholser and Cruess (**4**) in 1923 reported that the organic peroxide of fresh apple juice was temporarily inactivated by 20 minutes heating at 73.5° C. and the peroxidase by 20 minutes at 90° C. While the inactivation of fruit catalase by heat was not investigated by us except incidentally, it is of interest to mention the results reported by Morgulis, Beber and Rabkin (**3**) who found that the pH value of the medium exerted a marked effect on the temperature required for inactivation of catalase from beef kidneys.

Gallagher (**2**) has reported on the behavior of the peroxidase of the mangold after heating at 100° C. but gives no data on the effect of hydrogen-ion concentration. Cruess and Fong (**1**) have reported preliminary observations on the effect of pH value on the inactivation temperature of oxidase in certain fruit juices.

Procedure

In the experiments reported upon in the present paper we have attempted to determine the effect of pH value on the temperature required for complete inactivation of the peroxidase and organic peroxide of certain fruits, and the effect of pH value on the weakening action of heat on fruit peroxidase at certain temperatures below the inactivation temperature.

In one series of experiments the natural fresh juices were used; in a second series the oxidase was precipitated by alcohol and purified by redissolving in water and reprecipitation with alcohol or acetone. Usually three precipitations were used. The purified oxidase was dissolved in water and diluted to the volume of the original juice.

The natural juices and the oxidase solutions were brought to various pH values and heated at various temperatures differing by 5° C. On cooling, the heated liquids were tested for peroxidase by the addition of H_2O_2 and an oxidase indicator, usually benzidine and guaiac. Very acid or alkaline solutions, after heating, were adjusted to a pH value in which the indicators used were operative before the tests were made. For the complete oxidase, *i.e.*, peroxide and peroxidase, the indicator (usually benzidine) was added without H_2O_2; a positive reaction indicated the presence of organic peroxide

as well as organic peroxidase. In some experiments the approximate inactivation times of the catalase at various temperatures were also determined.

Inactivation temperature

In preliminary experiments with prune juice of pH 5.0, pear juice of pH 4.5 and peach juice of pH 3.5 it was found that the peroxidase, catalase and organic peroxide of the peach juice were inactivated at a considerably lower temperature than in the prune and pear juices. It appeared that the lower pH value of the peach juice might account for the observed difference in resistance of the oxidase and catalase to heat.

Portions of these three juices and of fresh fig juice were brought to various pH values. The approximate inactivation temperatures of the peroxidase, organic peroxide and catalase were determined and found to depend upon the pH value of the juices and not upon the variety of fruit used.

In order to test this finding further several varieties of fresh fruit juices were brought to various pH values by the addition of citric or tartaric acid, NaOH or $NaHCO_3$ as required. Portions of 10 cc. were heated for 10 minutes at 5° C. intervals of temperature, *e.g.* 40° C., 45° C., 50° C., etc., and the approximate inactivation temperatures determined. In other experiments the peroxidases of apricots and of peaches were precipitated with ethyl alcohol and purified by reprecipitation by alcohol from water solution. The purified peroxidases were dissolved in water, brought to various pH values, and the approximate inactivation temperatures determined as previously described.

In order to conserve space a summary only of the data will be given.

Prune[1] peroxidase in the natural juice at pH values of 2.4–2.8 was inactivated at less than 50° C.; that of pH 2.0 was inactivated at room temperature; at pH 3.1 it was inactivated at 70° C.; at pH 4.5, 90° C. was required for inactivation; at pH 5.0, 95° C. and pH 6 and 7, 100° C.

The peroxidase from green dates was somewhat more resistant. The peroxidase in tomato juice required the following temperatures for inactivation at the pH values indicated: 2.0, room temperature; pH 2.8 less than 50° C.; pH 3.0 and 3.4, 80° C.; pH 3.6, 3.8, 4.0 and 4.5, 95° C.; 6.0 and 7.0, 100° C.; and pH 8, 95° C. The peroxidase in fresh apricot juice required the following temperatures for inactivation at the pH values indicated: pH 2.6, approximately 40° C.; pH 2.8, 40–50° C.; pH 3.0 and 3.2, 60° C.; pH 3.5, 80° C.; pH 3.6, 90° C.; pH 4.0, 93° C.; pH 7.0, 98–100° C.; and at pH

[1] In this and following experiments the temperatures given are approximate as the temperature interval was 5° C. Thus an "inactivation temperature" of 50° C. signifies that the enzyme survived 45° C. but not 50° C.

10, 70–75° C. The purified peroxidase from apricots gave results similar to those given for the natural juice, although the purified oxidase was noticeably less resistant than that in the natural juice.

For the peroxidase in the natural juice expressed from ground peaches previously held in freezing storage, the following approximate inactivation temperatures were obtained: pH 2.6, 40° C. or less; pH 2.8, 50° C.; pH 3.0, 60° C.; pH 3.2, 80° C.; pH 4.0, 95° C.; pH 7.0, 100° C.; pH 8.0, 90° C.; pH 10, 85° C.; pH 12, room temperature. With the purified peroxidase the inactivation temperatures were somewhat lower, in general about 5° C. lower than for the peroxidase in the natural juice. The juices of bananas and pears behaved similarly to those of apricots and peaches.

The peaches exhibited a much stronger peroxidase reaction than did the apricots, which may account for the greater observed resistance to heat of the peroxidase in the peach juice.

The organic peroxide in the juices of peaches, apricots, pears, and prunes generally exhibited a heat resistance similar to that for the peroxidase; although in some instances probably owing to its low concentration in these cases, it was slightly less resistant to heat than the peroxidase. Whether it was destroyed by heat or whether the O_2 of the peroxide complex was merely consumed was not determined definitely, although allowing the heated juices to stand 24 hours did not result in reformation of the organic peroxide. In some cases there was evidence that, after 24–36 hours, the apparently inactivated peroxidase had regained some of its original power to produce a positive test (faint) with benzidine plus dilute H_2O_2.

All of the juices tested at pH 2.0 were inactivated at room temperature; that is, after addition of $NaHCO_3$ to juice previously of pH 2.0, and thus bringing it to pH 7–8, tests with H_2O_2 plus benzidine and guaiac tincture were negative; similarly, with 24 hours' contact, there was found to be inactivation of the peroxidase at pH 12 at room temperature.

Our data indicate that the inactivation temperature of the peroxidase in the juices of apricots, peaches, pears, prunes, bananas, tomatoes, avocados and dates is markedly affected by the pH value of the juice. The resistance increased in general from a pH value of pH 2.0 to 7.0 and decreased from pH 8.0 to 12.0. Between pH values 3.0 and 4.5 the resistance to heat increased very rapidly, and from 4.5 to 7 slowly, or remained fairly constant. Temperatures above 100° C. were not used.

The organic peroxide behaved similarly to the peroxidase except for the fact that at pH values above 8 the organic peroxide appeared to have decomposed because of the alkalinity of the medium and failed to give positive tests even in the unheated samples.

Effect of pH value on weakening action of temperature on fruit peroxidase

Apricot juice was brought to pH values of 3.7, 5.2, 7.8 and 9.2. 100-cc. portions of juice of each pH value were heated to 50, 60, 78, 95 and 100° C. for 5 minutes. After heating and cooling, the juices were brought to approximately the same pH value. They were divided into 10-cc. portions, and a small amount of 0.3 per cent. H_2O_2 and the following peroxidase indicators were added: pyrogallol, hydroquinon, guaiacol, benzidine, p-amino-phenol, o-amino-phenol and alpha naphthol. Using the unheated samples as checks, the depth of color of the various indicators was measured comparatively after 5 hours and after 48 hours in a Klett colorimeter.

At pH 9.2 some of the indicators oxidized spontaneously even in the boiled samples. Guaiacol and benzidine were not so affected. The data for the benzidine indicator is given in table I. The untreated juice of pH 7.8 was used as the basis of comparison. As the readings of the colorimeter vary inversely with the depth of color, the reciprocals of the readings multiplied by 1,000 are given in the table. These are calculated to the basis of 100 for the untreated check of pH 7.8. The readings given were taken after 5 hours' standing; the readings taken at this time appeared to be more definite than those taken at shorter or longer intervals.

TABLE I

COLORIMETER DETERMINATION OF PEROXIDASE, WITH BENZIDINE INDICATOR

TEMPERATURE AT WHICH SAMPLE WAS HEATED	pH VALUES AND COLORIMETER READINGS			
	pH 3.7	pH 5.2	pH 7.8	pH 9.2
Room	110	140	100	91
50° C.	100	110	100	82
60° C.	91	110	100	....
78° C.	0	96	55	53
95° C.	0	10	50	53
100° C.	0	0	0	0

The results with guaiacol were similar to those reported above for benzidine, although owing to the fact that it is not so easily oxidized as benzidine the apparent inactivation temperatures were lower than those indicated for benzidine. In general the decrease in activity of the peroxidase at a given pH value with progressive increase in temperature appears to be somewhat abrupt instead of gradual. However, there is some evidence of a gradual reduction in activity. At pH 7.8, 9.2 and 3.7 the oxidase was apparently

somewhat weakened by the reaction of the medium, or the benzidine pH optimum lies near 5.2. At pH values of 5.2 and 7.8 there was also considerable indication, with hydroquinon and pyrogallol indicators, that the decrease in activity of the oxidase with increase of temperature of heating was gradual.

Summary

The temperature required for the inactivation of the peroxidase of apricots, pears, peaches, prunes, figs, lemon peel, tomato, banana and dates was found to vary with the hydrogen-ion concentration. Resistance to heat was greatest in the range of pH 5 to 7. Resistance decreased with decrease in pH value between pH 5 and 2, and decreased with increase in pH value between pH 8 and 12. At pH 12 and pH 2–2.2 the peroxidase was inactivated at room temperature in 24 hours or less.

The organic peroxide behaved similarly to the peroxidase between pH 2 and 7. At pH values above pH 8 the organic peroxide failed to give positive tests. There was considerable evidence that the inactivating effect of temperature at any given pH is progressive rather than abrupt.

FRUIT PRODUCTS LABORATORY,
UNIVERSITY OF CALIFORNIA.

LITERATURE CITED

1. CRUESS, W. V., and FONG, W. Y. Observations on fruit oxidase. Fruit Products Jour. **6**: 13–15. 1926.
2. GALLAGHER, P. H. Peroxidase. Observations on the thermostability of the peroxidase of the mangold. Biochem. Jour. **18**: 39–46. 1924.
3. MOUGULIS, S., BEBER, M., and RABKIN, I. Studies on the effect of temperature on the catalase reaction. Jour. Biol. Chem. **68**: 521–533. 1926.
4. OVERHOLSER, E. L., and CRUESS, W. V. A study of the darkening of apple tissue. California Agr. Exp. Sta. Tech. Paper 7. 1923.

JOANNES BAPTISTA VAN HELMONT
1577–1644

BRIEF PAPERS

JOANNES BAPTISTA VAN HELMONT

(WITH ONE PLATE AND TWO FIGURES)

Plant physiology owes to the alchemist, VAN HELMONT, plate V and fig. 2, the introduction of the experimental method which he first used to determine the substances actually entering into the composition of plants. VAN HELMONT was an investigator of highest rank and the most eminent chemist of his time. His early training in medicine gave him an interest in the physiology of both plants and animals. In his study of the composition of organisms he came to the conclusion that water was the chief constituent. He arrived at this opinion both from experiments on the production of water by distillation in the analysis of organisms, and from his famous experiment on the production of plant substance by synthesis from water. The proof in his experiment rests on his belief that he had excluded all other sources of nutriment by checking the weight of soil substances absorbed. One understands quite readily, therefore, how he became convinced of the transformation of water into plant substance. However erroneous his deduction has proven to be, his experiment was well planned and his conclusion was logical on the basis of the information existing at that time on the nature of gases and the composition of the atmosphere. His son, FRANCISCUS MERCURIUS VAN HELMONT (see figures 1 and 2) has described this experiment in the collected works of the elder VAN HELMONT, which he published in 1648 under the title "*Ortus Medicinae vel Opera et Opuscula Omnia*" as follows:

"I took an earthen vessel in which I put 200 pounds of soil dried in an oven, then I moistened with rain water and pressed hard into it a shoot of willow weighing five pounds. After exactly five years the tree that had grown up weighed 169 pounds and about three ounces. But the vessel had never received anything but rain water or distilled water to moisten the soil when this was necessary, and it remained full of soil, which was still tightly packed, and, lest any dust from outside should get into the soil, it was covered with a sheet of iron coated with tin but perforated with many holes. I did not take the weight of the leaves that fell in the autumn. In the end I dried the soil once more and got the same 200 pounds that I started with, less about two ounces. Therefore the 164 pounds of wood, bark, and root arose from the water alone." From the translation in E. J. RUSSELL, "Soil Conditions and Plant Growth" of his "*Opera Omnia Complexionum atque Mistionum Elementalium Figmentum.*"

VAN HELMONT was born of a noble Brabantine family at Brussels in 1577 and died on his estate at Vilvorde near Brussels in 1644. His life for the greater part was that of a scholar, working in quiet. His great knowledge of chemistry brought him many enticing offers from princes for his

FIG. 1. FRANCISCUS MERCURIUS VAN HELMONT. From the painting by Sir Peter Lely, in the National Portrait Gallery, Trafalgar Square, London. Published by permission of the British Museum.

services, but owing to his excellent family connections and brilliant circumstances, he was able to devote without interruption his great energies to experiments in his own private laboratory. At an early age he studied

philosophy and theology and became engrossed in mysticism and the alchemistic writings. His unusually keen mind, however, soon recognized the weaknesses of the Aristotelian doctrine, that matter was composed of four elements: earth, fire, air, and water. He denied that fire could be of a material nature. It is hardly possible that he regarded air as a chemical element, for he was the first to characterize gaseous substances as differing

FIG. 2. Photograph from the memorial window in the alchemists' laboratory of the Deutsches Museum, München, Deutschland. The armorial bearings of the VAN HELMONT family from this window has been included.

in properties. He described hydrogen and methane as peculiar varieties of air. He showed that carbon dioxide could be produced from limestone or ashes by treatment with acids, from burning coal, and in the fermentation of wine and beer. He termed carbon dioxide "gas sylvestre" because he could not condense it. He introduced the term "gas," possibly having in mind the production of such substances during fermentation, the Dutch verb which means "to ferment" being "gisten."

He considered fermentation as the principal cause of digestion. He held that the acidity of the medium regulated the action of the body fluids, and showed that the acid of the gastric juice favored digestion. He compared the interactions of various digestive juices which mingle with one another with similar reactions of solutions outside of the organism, and thus laid a foundation for chemical physiology. He was a real contributor of fundamental research in a dark age of science.

A biography was published in 1922 by H. Stanley Redgrove and I. M. L. Redgrove, under the title "Joannes Baptista van Helmont."—R. B. HARVEY, *University of Minnesota.*

NOTES

Des Moines Meeting.—The program committee, under the chairmanship of Dr. W. E. Loomis, and with the assistance of the officers of the American Society of Plant Physiologists, is engaged in arranging the program for the Des Moines meeting. The sessions will be held on December 30 and 31, 1929, and January 1, 1930. Members are urged to attend this meeting if at all possible.

With the increase in size of the Society the Des Moines meeting should be the best yet held. A joint meeting with the Horticultural Society is to be held on Tuesday morning, December 31; and Wednesday afternoon, January 1, 1930, is reserved for a joint session with Section G of the American Association for the Advancement of Science.

Special attention is being given to the annual dinner, which has been planned for the evening of January 1, 1930, if no serious conflicts arise, and it is hoped that all members in attendance will reserve this date. Two special features of the dinner program will be the first award of the Stephen Hales Prize, and the fourth award of the Charles Reid Barnes Life Membership. There will also be a brief talk by the President of the Society, and probably an illustrated address by Dr. L. G. M. Baas-Becking, Director of the Jacques Loeb Laboratory of the Hopkins Marine Station of Stanford University, on the work of the Laboratory.

Let us all cooperate to make the coming Des Moines meeting a live and enthusiastic affair, and a real contribution to the cause of plant physiology. There are two things that each member can do to make the meeting a great success. One is to attend the meeting; the other is to take active part in the discussion of the papers presented. The Society has already established fine traditions for its meetings, and it hopes to maintain the standards thus established.

Foreign Members.—Plant physiologists residing in foreign lands are eligible to membership in the American Society of Plant Physiologists, and our foreign members are invited to interest their friends and colleagues in such membership whenever it is possible. The dues are only slightly larger than for North American members, to cover a part of the additional postage. Those who desire membership should address Dr. H. R. Kraybill, Purdue University, Lafayette, Indiana.

Portrait of Van Helmont.—Again we are pleased to be able to announce that copies of the J. B. Van Helmont portrait are available for those who desire them for framing. The group of four, Timiriazeff,

SACHS, PFEFFER, and VAN HELMONT, may be obtained for 48 cents, postage paid. Orders may be sent to the editor of PLANT PHYSIOLOGY, with stamps or cash accompanying the order.

Manuscripts.—Members of the Society are invited to assist the Editorial Board to secure a larger volume of high class manuscripts for publication in PLANT PHYSIOLOGY. During the last year the manuscripts have been used up very closely each quarter, and with the October number we present all of the material which was available on September 15, 1929.

The Society is financially able to publish a somewhat larger volume than the current one, but cannot do so unless the manuscripts are forthcoming, and will not do so unless they are of high enough quality. Such assistance as the members may be able to render will be greatly appreciated.

Errata.—A few errors have been found in this volume of PLANT PHYSIOLOGY, and the corrections are placed at the close of the Table of Contents. It is suggested that corrections be entered at the places where the errors occur. The occasional assistance of readers in detecting such mistakes is greatly appreciated.

Fan Chi Kung.—It is with much regret that we announce the death of another member of the Society. The account of Mr. KUNG's life which is here presented has been furnished by Prof. LOUIS DEVRIES, adviser to foreign students at Iowa State College, Ames, Iowa.

Mr. FAN CHI KUNG was born on August 24, 1900, at Chengtu, Szechwan, China. Both of his parents died early and he was brought up by his grandfather, who died when he was 14 years old. He was then supported by his uncle. He entered the Junior Middle School of the Tsing Hua College, Peking, in the fall of 1915. He was graduated from the Tsing Hua College in the spring of 1923. During those eight years in Tsing Hua, he was Class President once, and held many other offices at other times. He was a member of the Executive Committee of the Tsing Hua Students' Association during the Student Movement in 1919. He came to the United States in the fall of 1923, and after staying in Cornell University for a time, he was transferred to Iowa State College, where he took up pomology, and from which institution he graduated in the summer of 1926.

Following his graduation at Iowa State College, he entered the University of Chicago, and spent a year as a postgraduate student in Plant Physiology. During the fall and winter of 1927 he was engaged in a study of the fruit industry in California, and in the spring of 1928 came back

to Iowa State College as a graduate assistant in the Pomology Section of the Iowa Agricultural Experiment Station, which position he held at the time of his death, which resulted from a car accident in the evening of July 4, 1929. He died that same night at 11:00 P.M. in the College Hospital. Burial took place in the College Cemetery.

During these few years in the United States he was Secretary of the U. S. Branch of the China Agricultural Association. He was also elected President of the Ames Cosmopolitan Club.

Physiological Basis of Drought Resistance.—In noticing the excellent work of MAXIMOW in the July number of PLANT PHYSIOLOGY, an unfortunate error was made in quoting the price. The book is published under the title "The plant in relation to water," is handled in this country by the Macmillan Co., and the price is $6.50. The editor regrets the mistake. The book is worthy of a place in every physiologist's library.

Outlines of Biochemistry.—A book of unusual value to students of the biological sciences has been prepared by Dr. ROSS AIKEN GORTNER, Professor of Agricultural Biochemistry at the University of Minnesota. The "Outlines of Biochemistry" is the outgrowth of the work of the Division of Agricultural Biochemistry over a period of nearly 20 years, and represents a rich harvest of experience in handling this subject for students of the non-medical biological fields. It is particularly fortunate that the final choice of material and manner of presentation rested with Dr. GORTNER, for he is not only an active investigator with years of first hand experience with the problems of biochemistry, but is also an able and inspiring teacher, who understands the importance of clear exposition. It was the writer's privilege and pleasure several years ago to attend many of the lectures which are now summarized in this book, and a great service has been rendered in giving them permanent form.

There are seven sections to the book, the first of which, consisting of ten chapters, deals with the colloid state of matter. This section occupies almost 300 pages, and the author is particularly at home in connection with the colloidal properties of matter which are of greatest interest to biological students. The succeeding sections consider the proteins; carbohydrates and allied compounds; tannins; plant pigments; fats, lipides, and essential oils; and the biocatalysts. Following the 734 pages of text, there are general references to the literature of each section, an author index containing more than a thousand names, and an ample subject index, bringing the volume to 793 pages. Every chapter is replete with interest. The plant physiologist will be especially pleased at the amount of material

that applies more or less directly to his field. The publisher's list price is $6.00, and orders for the book should be addressed to John Wiley and Sons, New York.

Electrostatics in Biochemistry.—With the title "Elektrostatik in der Biochemie," a course of lectures offered at the University of Basel in October, 1928, is now issued in book form. They were originally published in the Kolloidchemische Beihefte, Vol. 28, Hefts 7–10. In addition to the introductory lecture and the final section of discussion, there are 18 lectures, dealing with electrostatics, electric potentials and their measurement, making of microelectrodes, pH determinations, vital staining, dispersity and particle size, dielectric constants, etc. Fourteen of the lectures are by members of the University of Prag, two from Graz, two from Basel, and one from Leipzig. It furnishes a satisfactory introduction to the field of electrostatics in biology. The publisher is Theodor Steinkopff, Dresden and Leipzig.

Physical Components of Plant Transpiration.—The second monograph from the field of scientific botany is entitled Die physikalische Komponente der pflanzlichen Transpiration. The author is Dr. A. SEYBOLD, of Köln, and the publisher Julius Springer, Berlin. This little monograph, dedicated to the memory of SACHS, presents the subject of transpiration in four chapters. The first deals with the physical basis of transpiration, evaporation in moving and still air, diffusion through models and porous systems, theory of psychrometers, etc. The second chapter takes up the quantitative aspects of water loss, with special reference to different types of plants. The third chapter considers the energy changes involved in the process, and the final chapter is a critical consideration of SCHIMPER'S theory of restricted transpiration of xerophytic plants. A concise summary of 7 pages concludes the work. It is a good summary for those interested in the problems of water loss from plants. The price in brochure form is RM 26.

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